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(54) CONVERTIBLE MICROEMULSION FORMULATIONS

KONVERTIERBARE MIKROEMULSIONSVERBINDUNGEN

FORMULATIONS DE MICROEMULSIONS A INVERSION DE PHASE

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- **CHEM. PHARM. BULL., vol. 31, no. 8, 1983 pages 2786-2792, AKIRA TAKAMURA ET AL 'Effect of Homogenization Conditions on the Physicochemical Properties of Emulsions Bases'**
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Description

[0001] This invention relates to microemulsions, and methods of making and using the same. More particularly, it relates to certain unique microemulsion formulations which are phase reversible (i.e., "convertible" as defined below), methods for making and storing them, and their use in administering drugs, proteins, and like biologically-active materials, including therapeutically-active ones.

[0002] As used herein, the microemulsions of this invention are self-emulsifying stable dispersions of oil and water, stabilized by interfacial films of surface-active molecules. These microemulsions are also characterized by their small average particle sizes, generally less than about 0.1 micron, by their wide range of temperature stability, typically from about 5°C to 50°C, and they appear to be thermodynamically-stable, i.e., stable indefinitely over this range. They are also relatively insensitive to the pH or ionic strength of the aqueous internal phase.

[0003] These microemulsions are further characterized in that they form spontaneously without the need of high shear equipment, as distinct from conventional emulsions (macroemulsions) which must be prepared by the input of significant amounts of energy, and which are thus subject to extremes of temperature, pressure, and shear, resulting in damage to the contents of the emulsion. For further discussion of these systems, see "Microemulsions," M. Kahlweit, *Science*, 240:617-621 (1988).

[0004] By the term "convertible" or "phase reversible", as used herein to describe the microemulsions of this invention, is meant a microemulsion formulation capable of being changed from a water-in-oil (w/o) system to an oil-in-water (o/w) system by the addition of water to the former, as described in further detail below.

[0005] Also, "conversion," as used herein, is intended to define in particular the reversal of a w/o emulsion to form an o/w emulsion, as distinct from the term "inversion", as used in the art, which describes principally the change of a w/o emulsion to a water-in-oil-in-water (w/o/w) formulation.

[0006] The Kirk-Othmer Encyclopaedia of Chemical Technology, 3rd Edition, vol. 8, 1979, page 908 discloses that the inversion of an emulsion is dependent on the hydrophilic-lipophilic balance (HLB) of the surfactant.

[0007] The preparation and use of microemulsions in the formulation of drugs, proteins, and the like are known in the art. See, for example, U.S. Patent 3,989,843, which discloses the application of microemulsions to medical formulations. Also, in *Eur. J. Biochem.*, Samama et al., 163(3):609-617 (March 16, 1987) describe liver alcohol dehydrogenase in ionic w/o microemulsions, while Lee et al. describe the extraction of epoxide cyclase, using various ionic microemulsions, in *FEBS Lett.*, 244(2):347-50 (Feb. 27,1989). In each case, however, there is no teaching or suggestion that these microemulsions are phase reversible.

[0008] U.S. Patents 4,931,210; 4,857,506; 4,714,566; and 4,590,086, on the other hand, disclose methods of preparing water-in-oil emulsions which are then inverted to form well-known water-in-oil-in-water phase (w/o/w) emulsions. These complex preparations, however, are macroemulsion formulations requiring high shear energy to prepare, and the resulting product is a w/o/w emulsion which actually comprises a w/o emulsion mixed into an aqueous phase in such a way that the first internal aqueous phase does not mix with the second continuous aqueous phase.

[0009] Emulsion systems for delivery of lipophilic agents via oral, parenteral, or local cutaneous administration and for transdermal delivery of the polypeptide hirudin are disclosed in U.S. Pat. No. 4,719,239 to Muller et al. Microemulsion systems containing drugs having a good hydrophilic/lipophilic balance for transdermal delivery are disclosed in GB Application 2,098,865. These references fail to disclose the use of a water-in-oil microemulsion for the mucosal delivery of a water-soluble active agent, such as proteins and peptides.

[0010] Emulsion systems have also been used as vaccine adjuvant systems, particularly water-in-oil emulsions. The strength of the immune response and the speed with which it is evoked can be modified by the nature of the liquid matrix of the vaccine. One widely-used example of such a system is Freund's adjuvant, which consists of paraffin oil and a surfactant, mannide mono-oleate. These adjuvant emulsions, due to their thermodynamic instability, must be emulsified with a solution containing the immunogen just prior to injection of the vaccine. In addition, the paraffin oil in the adjuvant can lead to inflammation of the injection site and formation of granulomas. These two effects are greatly enhanced if immune stimulators are also employed. The oil and immune stimulators are helpful, however, in that they stimulate immune response by enhancing the activity of macrophages. These macrophages engulf the emulsion droplets and process the immunogen at the site of the injection. It would, therefore, be beneficial to be able to produce a vaccine adjuvant system which has a prolonged stability and thus, a prolonged shelf life in its prepared microemulsion state, and which can be formulated with a biodegradable oil which would not stimulate granuloma production.

[0011] There is a continuing need for new and improved delivery systems for biologically active materials. Many of the therapeutic agents emerging from the biotechnology revolution, as well as some older drugs such as insulin and calcitonin, consist of large-molecule proteins. These drugs must now be injected into the patient because they are unable to survive the digestive process and do not readily pass through the mucosal lining of the gastrointestinal tract and enter the bloodstream. A new drug delivery system that would enable proteins to enter the bloodstream through, for example, the lining of the digestive system would be of great benefit.

[0012] Improved drug delivery systems could also provide much improved convenience for patients. For example, cal-

citonin is a generic peptide hormone used for treatment of osteoporosis and other diseases involving bone loss. Osteoporosis affects 24 million Americans, including 2/3 of the women past menopause. Currently, most calcitonin is delivered by injection. Calcitonin treatment for osteoporosis requires long-term administration with low but frequent doses of the drug. An oral or suppository formulation of calcitonin would offer great advantages to patients underoing such treatments.

[0013] In accordance with the present invention, there are now provided compositions as defined in the accompanying claims comprising a highly stable water-in-oil microemulsion containing biologically, including therapeutically, active water-soluble materials in an internal aqueous phase, which water-soluble materials are controllably releasable when needed just prior to administration by the ready conversion of the microemulsion into an oil-in-water emulsion by the addition of water to form a continuous aqueous phase.

[0014] The invention also relates to the use of such microemulsions in the administration of biologically and therapeutically active water-soluble materials, as defined in the accompanying claims.

[0015] One advantage of the invention is the storage or maintenance of materials, such as proteins and peptides, in a solubilized state at temperatures or conditions at which they would otherwise be unstable. For example, it has been found that some proteins can be stored dissolved in the aqueous phase of the w/o microemulsions at temperatures at which the protein would be unstable if stored merely as an aqueous solution. Such proteins may be stored in a w/o microemulsion of this invention until ready to be used, at which time water is then added until an o/w emulsion has formed, which emulsion is then administered orally or by injection. Also, the stored w/o microemulsion can be administered to the body wherein it is converted to an o/w emulsion by the addition of bodily fluids. In this manner, storage problems are lessened or eliminated.

[0016] Typical of the storage times for drugs, proteins, and the like, which may be achieved with the compositions of this invention, are times anywhere from 1 to 48 hours, preferably 16-24 hours up to several, i.e., 3-12, weeks or months, at temperatures of from about room temperature, i.e., 20°C, up to the temperature where the microemulsion breaks, generally in the range of 50-70°C, preferably below 40°C. Temperatures below room temperature can, of course, be used.

[0017] In a further aspect of this invention, it has been found that, unexpectedly, if a w/o microemulsion of this invention containing, for example, a water-soluble drug in the internal aqueous phase, is administered directly to the body of animals, including humans, the body fluids themselves are sufficient to convert the w/o microemulsion to an o/w emulsion, thereby slowly releasing the drug *in situ*. This is particularly advantageous over pre-conversion with water in that because body fluids are employed, the total volume of liquid administered is smaller. This method is particularly useful in administration into the colon or intestines of such drugs as peptides, proteins, or other molecules with bonds that are readily attacked by enzymes, where the oil protects the drug in the intestines until it is slowly released as the body fluids convert the emulsion. In the case of calcitonin, for example, if it is administered into the colon as just an aqueous solution, colon enzymes destroy the drug before it is absorbed, whereas with the microemulsion formulations of this invention, the calcitonin is protected from the enzymes until it is slowly released by hydration within the body.

[0018] In one particular embodiment of the present invention the w/o microemulsion system is formulated such that, upon conversion with additional water, an o/w microemulsion is formed. Such a system is advantageous in that the converted system has a small particle size. In another embodiment of the present invention, the microemulsion system is formulated as a solid at room temperature which has surprisingly been found to enhance drug uptake and activity for gastro-intestinal delivery.

[0019] A particular embodiment of the present invention is the use of a w/o microemulsion as a vaccine adjuvant system. The immunogen is carried in the aqueous phase of the microemulsion adjuvant system, which when introduced into the body and contacted with aqueous bodily fluids, undergoes conversion to form an oil-in-water emulsion.

[0020] "Administration to the body", as used herein for systems that convert to macroemulsions, includes any non-intravenous method such as intramuscular, subcutaneous, oral, rectal, or peritoneal means. More specifically, the w/o microemulsion is administered parenterally, enterally, or via any other mucous membrane. Systems that convert to microemulsions can also be administered intravenously and intraarterially.

[0021] In yet another embodiment of this invention, it has been determined that these w/o microemulsions may also be used to formulate topical salves which are highly advantageous in that they remain moist on the skin for long periods of time without drying and crumbling.

Brief Description of the Drawings

[0022]

Fig. 1 is a phase diagram of an embodiment of the present invention depicting the water-in-oil microemulsion region wherein the oil is Captex 200, the aqueous phase is a 0.9% wt. NaCl aqueous solution, and the surfactant mixture is Capmul MCM:Myverol 18-92:Cremophor EL.

Fig. 2 is a phase diagram of an embodiment of the present invention depicting the water-in-oil microemulsion region wherein the oil is Captex 200, the aqueous phase is 0.9% wt. NaCl aqueous solution, and the surfactant mixture is Capmul MCM:Centrophase 31:Tween 80.

Fig. 3 is a phase diagram of an embodiment of the present invention depicting the water-in-oil microemulsion region wherein the oil is Captex 200, the aqueous phase is 0.9% wt. NaCl aqueous solution, and the surfactant mixture is Capmul MCM:Centrophase 31:Cremophor EL.

Fig. 4 is a phase diagram of an embodiment of the present invention depicting the water-in-oil microemulsion region wherein the oil is Whitepsol H-15, the aqueous phase is a 20% wt. Sorbitol in 0.9% wt. NaCl aqueous solution, and the surfactant mixture is Capmul MCM:Myverol 18-92:Tween 80.

Fig. 5 is a phase diagram of an embodiment of the present invention depicting the water-in-oil microemulsion region wherein the oil is MYVACET 9-45K, the aqueous phase is 0.9% wt. NaCl aqueous solution, and the surfactant mixture is Capmul MCM:Myverol 18-92:Cremophor EL.

[0023] The biologically active material composition of this invention comprises, at a minimum, (1) an aqueous phase; (2) a pharmaceutically-acceptable oil, or mixtures thereof; (3) an oil-dispersible surfactant, or mixtures thereof; and (4) a water-soluble biologically active material or combination of materials, as defined in the accompanying claims In addition, there may optionally be included such other adjuvants as stabilizers, coloring agents, oil soluble drugs and the like. Each of these components and adjuvants must be suitable for use in the subject and will usually be food grade and/or pharmaceutically-acceptable materials. Any drugs will be present in therapeutically-effective amounts. The compositions of the present invention are biologically compatible water-in-oil (w/o) microemulsions. These compositions are biologically compatible in that they are non-toxic and contain biodegradable or non-absorbable materials. By non-toxic it is meant non-toxic dependent upon the route of administration to a subject, in that the toxicity of one route may not be equivalent to that of another route.

[0024] The microemulsions of the present invention are created by the interplay between the surfactant or mixture of surfactants and the oil and aqueous phases. The surfactant or mixture of surfactants preferably have a hydrophilic-lipophilic balance (HLB) within a specified range. By "hydrophilic-lipophilic balance" is meant an empirical quantity, on an arbitrary scale, which is a measure of the polarity of a surfactant or mixture of surfactants. See P. Becher et al., "*Non-ionic Surfactant, Physical Chemistry*," Marcel Dekker, NY (1987), pages 439-456. It is a widely known and used term. The w/o microemulsions can be solids including semi-solids, gels, or liquids at room temperature.

[0025] More particularly, the amount of the components should be such that the biologically-active material comprises from 10^{-9} to 100 weight/volume %, based on the volume of the aqueous phase. Generally, in the microemulsion system, the aqueous phase ranges up to 60 volume percent; the oil content ranges from 5 to 99, preferably from 10 to 99 volume percent; the surfactant content ranges from 1 to 70 volume percent.

[0026] The water content in the w/o microemulsions is up to 20 volume percent, preferably up to 30 volume percent, most preferably up to 40 volume percent, and in some cases as high as 60 volume percent of the microemulsion. In a preferred high aqueous phase content w/o microemulsion system, the aqueous phase content ranges from 20 to 60 volume percent, preferably from 30 to 60, most preferably 40-55%; the oil content ranges from 5 to 50 volume percent, preferably from 5 to 40, most preferably 5-15%; the surfactant content ranges from 5 to 75 volume percent, preferably from 20 to 65, most preferably 40-50%. In a preferred low aqueous phase content w/o microemulsion system, the aqueous phase should comprise no more than 20%, preferably the aqueous phase content ranges from 0.1 to 20 volume percent, most preferably 0.1-15%; the oil content ranges from 30 to 99 volume percent, preferably 50-90%; the surfactant content ranges from 1 to 70 volume percent, preferably 2-50%. When the aqueous phase of the w/o microemulsion is below 20% volume, it is preferred to have a ratio of oil phase to low HLB surfactant, HLB below 8, preferably below 5, of at least 6:1, and preferably at least 10:1. The water component of the aqueous phase can be partially or fully replaced by the incorporation of another polar, biologically compatible solvent such as polyhydrolic alcohols having at least 2 hydroxyl groups, glycerol, propylene glycol, and mixtures thereof, however it is preferred to have the aqueous phase consist of at least 30%, and most preferably 50% water. Thus, the term "aqueous phase" as used herein is intended to encompass a phase comprising water, such polar solvents, and mixtures thereof. The aqueous phase may comprise, in addition to water (or other polar solvent) and active material, such other adjuvants such as, but not limited to, stabilizers, coloring agents, modifiers, and the like, or salts (e.g., when saline is used).

[0027] The formulation of a microemulsion having a high aqueous phase content is preferred in those situations where the biologically-active material has a relatively low solubility in water or where a relatively high quantity of the biologically-active material is desired in the microemulsion system.

[0028] Adjuvants, such as preservatives, coloring agents, flavors or oil-soluble drugs, e.g., steroids, if any, should be included only in those amounts which will not adversely affect the novel properties of the microemulsion, generally in amounts of from 0 to 20% by volume, based on the total volume of the composition.

[0029] In the following description it will be understood that the nature of the oils and surfactants is not critical beyond those particular qualifications set forth below, and may generally be any such known materials conventionally employed

and which are accepted in the food and pharmaceutical industry.

[0030] The oil, or mixtures thereof, may be liquid at room temperature, although in some cases, mild heating of a solid oil to form a liquid is acceptable. If injection is the preferred route of administration, the oil should be liquid at room temperature. Heating of an oil that is solid at room temperature is desirable for formulations intended as suppositories, creams, salves, and in some cases as oral capsules. Illustrations of suitable oils for purposes of this invention include triesters of glycerol having from 9 to 83, preferably 20-60, carbon atoms, and diesters of propylene glycol having from 7 to 55, preferably 15-40 carbon atoms, most preferably propylene glycol esters of capric and caprlic acids having from 19 to 23 carbon atoms.. The triglycerides are further defined as short chain triglycerides having 9-15 carbon atoms, medium chain triglycerides having 21-45 carbon atoms, and long chain triglycerides having above 45 carbon atoms. Short chain and medium chain, and preferably short chain, triglycerides are preferred for liquid w/o microemulsion systems. The diesters of propylene glycols are further defined as short chain having from 7-11 carbon atoms, medium chain having from 15-31 carbon atoms, and long chain having above 31 carbon atoms. Examples of glycerol triesters include natural, edible oils such as canola, corn, olive, sunflower and coconut oils, triacetin, the decanoic acid esters, and chemically-synthesized oils such as 1 -oleyl-2,3-diacetyl glycerol. Diesters of propylene glycols include propylene glycol esters of capric and caprylic acids, such as Captex 200® (Karlshamns Lipid Specialities, Columbus, OH) and other ester groups as described above for glycerol.

[0031] As shown in the data below, it has been found in another embodiment that, surprisingly, when a mixture of an oil and mono and diglyceride surfactants, particularly Captex 200® and Capmul MCM®, manufactured by Karlshamns Lipid Specialities of Columbus, OH, as defined below, are used together, there is a significant enhancement in activity of the active ingredient. Therefore, depending upon the nature of the drug, mixtures of oils and mono and diglycerides may be preferred.

[0032] The surfactant, or more preferably, the mixture of surfactants, should be chosen from those having a resulting HLB value in the range of from 7 to 14, more preferably 8 to 13. When a mixture of surfactants is employed, while some of the components may have a value outside the desired range, e.g., below 5, it will be understood that by mixing in surfactants with HLB's greater than, e.g., 9, the resulting combined HLB value will be in the range of 7 to 14. Also, when a mixture is employed, it is desirable that at least one of these surfactants have a molecular weight of at least 500, although this weight is not critical. It has been found that although some protein and peptide delivery systems require the presence of certain surfactants, such as sterols or lecithin, the present w/o microemulsion systems do not require any particular surfactant or surfactant mixture, and can be essentially free, that is containing less than 0.05% wt. in the w/o microemulsion, of any of the listed surfactants. However, to promote bioavailability of the active agent, certain surfactants are preferred.

[0033] A mixture of surfactants is preferred when the w/o microemulsion has an aqueous phase content of greater than 20% by volume. The mixture includes a high HLB surfactant or mixtures of high HLB surfactants, having a HLB value of greater than 9 and preferably at least one surfactant having a HLB value greater than 12. In some embodiments having a relatively high aqueous phase content above 40% by volume, it is preferred to have at least one surfactant with a HLB greater than 15, and a low HLB surfactant having a HLB value below 5, which together have an average HLB value of from 7 to 14. Further, the surfactant should desirably be highly oil-soluble or oil-dispersible, and the ready addition of the surfactant to the oil thus makes for easier processing.

[0034] Surfactants which may be employed in our compositions include both ionic agents, i.e., cationic, anionic or zwitterionic, and non-ionic agents, or mixtures thereof. Examples of cationic surfactants include cetyldimethylethylammonium bromide, cetylpyridinium chloride and other salts of these surfactants.

[0035] Examples of anionic surfactants include C_{8-32} fatty acids and salts thereof; cholic acid and derivatives thereof such as deoxycholate, and its salts, ursodeoxycholic acid, and taurocholic acid; C_{8-56} diesters of tartaric acid; phospholipids such as phosphatidic acid and phosphatidyl serine; C_{5-29} monoesters of lactic acid; C_{8-20} sulfonates, including alkyl-, olefin-, and alkylaryl derivatives; tridecyl- and dodecylbenzene sulfonic acids; and C_{5-33} sarcosine and betaine derivatives.

[0036] Zwitterionics include such phospholipids as lecithin, phosphatidylethanolamine, and sphingomyelins.

[0037] Among the non-ionic surfactants which may be employed are ethoxylated castor oil; C_{5-29} mono-glycerides and ethoxylated derivatives thereof; C_{15-60} diglycerides and polyoxyethylene derivatives thereof having 1 to 90 POE groups; C_{10-40} esters (10-40 carbon atoms in the alcohol) of long chain fatty acids(fatty acids having 16 carbon atoms and above); C_{10-40} alcohols; sterols such as cholesterol, ergosterol, and C_{2-24} esters thereof; C_{8-96} ethoxylated fatty esters; C_{14-130} sucrose fatty esters; and C_{20-130} sorbitol and sorbitan monoesters, diesters, and triesters, and polyoxyethylene (POE) derivatives thereof having 0 to 90 POE groups, e.g., polyoxyethylene sorbitan monooleate, sorbitol hexaoleate POE (50). Of these, mono- and di-glycerides, or mixtures thereof, are preferred as low HLB surfactants and the sorbitol and sorbitan compounds as high HLB surfactants. More specifically, preferred low HLB surfactants include C_9 to C_{13} monoglycerides (HLB 4-7), C_{19} to C_{25} diglycerides of mono and poly unsaturated fatty acids (HLB 3-5), C_{15}-C_{23} diglycerides (HLB 4-6), and C_{35} to C_{47} diglycerides of mono and poly unsaturated fatty acids (HLB 2.5-4.5); preferred high HLB surfactants include ethoxylated castor oil (HLB 10-16) and the sorbitan surfactants with HLB from 10-

18. Short chain monohydroxyl alcohols, such as C_1 to C_6 are not employed as surfactants in these systems due to toxicity factors.

[0038] As stated above, the molecular weight of these surfactants is not critical, but it is desirable that at least one of them have a molecular weight of at least 500, more preferably greater than 750.

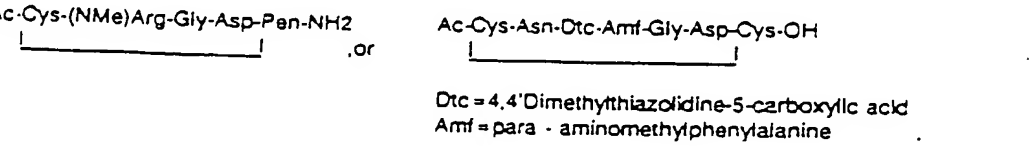
[0039] The water-soluble active material to be incorporated in the internal aqueous phase of the w/o microemulsion may be any biologically active material, particularly water-soluble proteins, peptides and other pharmaceutically-active compounds, i.e., drugs, and compounds which may have use as diagnostic agents. Vitamins and other food supplements which are not commonly defined as being "therapeutic" are not within the definition of the active agent. Illustrations of proteins which may be advantageously formulated, particularly for prolonged storage, include enzymes, such as horseradish peroxidase, alkaline phosphatase and derivatives thereof; and other unstable proteins which tend to undergo inactivation during storage at elevated temperatures, such a cytokines, hemoglobin, interleukins, and the like. Peptides including polypeptide hormones such as calcitonins, insulins, and the like are suitable for incorporation.

[0040] Other active agents that can be used in the w/o microemulsion system include peptides which may be satisfactorily employed include such pharmaceutically-active peptide drugs as desmopressin (1-desamino-8-D-arginine vasopressin). Drugs that can be employed in this system are water soluble drugs which are characterized by having low oral bioavailability. Examples of some of the drugs that can be employed include: anticoagulants, such as heparin or its derivatives; antimicrobials, such as penicillin G, carbenicillin, meziocillin and other poorly absorbed penicillin derivatives; cephalosporins, such as cephalothin, cefoxitin, cefotaxime and other molecules in this series normally administered by injection; antineoplastic drugs, such as fluorouracil, cytarabine, azauridine, thioguanine, vinblastine, vincristine, and bleomycin; anti-inflammatories, such as aurothioglucose and gold sodium thiomalate; and antiparasitic drugs, such as suramin and mebendazole.

[0041] Other active agents include RGD peptides, hematoregulatory peptides, vasopressin, collagenase inhibitors, angiotensin inhibitors, mammalian growth hormones, erythropoeitins, interleukins (e.g. IL-2, 3, 4 and the like), clotting factors (e.g. factors VII, VIII, IX) colony stimulating factors (e.g. G-CSF, GM-CS, M-CSF), hypothalamic releasing peptides (e.g. growth hormone releasing peptides, gonadotropin releasing factors), interferons, tissue plasminogen activators, atrial natriuretic peptides, tumor necrosis factor, antibodies, antibody fragments, clotting factors, dismutases, vaccine, immunoregulators, HIV protease inhibitors, neurotrophic factors (e.g. nerve growth factors), peptide and protein mimetics, and angiotensin II antagonists.

[0042] The present invention also provides for formulations incorporating small peptides, from 2 to 10, more preferably from 2 to 6 amino acid moieties. One group in particular, the fibrinogen receptor antagonists (RGD containing peptides) are tetrapeptides with an average molecular weight of 600. These peptide antagonists are highly potent platelet aggregation inhibitors at plasma levels as low as 1 pmol/ml. A preferred fibrinogen antagonist is the peptide cyclo(S,S)-N^{α}-acetyl-Cys-(N^{α}-methyl)Arg-Gly-Asp-Pen-NH_2 prepared by the method of Ali et al., published application EP-A-0 341 915. Also preferred is the peptide cyclo(S,S)-(2-mercapto)benzoyl-(N^{α}-methyl)Arg-Gly-Asp-(2-mercapto)phenylamide which may be prepared by the method disclosed in published EP-A-0423212, Application No. 90311537.6. The RGD peptides can generally be included into the microemulsion in an amount up to about 50 mg/ml of the aqueous phase.

[0043] Other fibrinogen antagonists useful in the present invention are those peptides disclosed in Pierschbacher et al., WO 89/05150 (US/88/04403); Marguerie, EP-A-0 275 748; Adams et al., U.S. Patent 4,857,508; Zimmerman et al., U.S. Patent 4,683,291; Nutt et al., EP-A-0 410 537; Nutt et al., EP-A-0 410 539; Nutt et al EP-A-0 410 540; Nutt et al., EP-A-0 410 541; Nutt et al., EP-A-0 410 767; Nutt et al., EP-A-0 410 833; Nutt et al., EP-A-0 422 937; Nutt et al., EP-A-0 422 938; Alig et al., EP-A-0 372 486 Ohba et al., WO 90/02751 (PCT/JP89/00926); Klein et al., U.S. Patent 4,952,562; Scarborough et al., WO 90/15620 (PCT/US90/03417); Ali et al., WO91/07429; peptide like compounds as disclosed in Alig et al., EP-A-0 381 033; and Alig et al., EP-A-0 384 362; and the cyclic RGD peptides:



[0044] Larger peptides/polypeptide also useful in the present invention are those disclosed in Pierschbacher et al., U.S. Patent 4,589,881 (>30 residues); Bittle et al., U.S. 4,544,500 (20-30 residues); and Dimarchi et al., EP-A-0 204 480 (>34 residues).

[0045] Also preferred are growth hormone releasing peptides, which are peptides generally of twelve amino acids or less and effect the release of growth hormone. The growth hormone releasing peptides can be used in an amount up to about 75 mg/ml of the agueous phase.

[0046] Exemplary of the class of growth hormone releasing peptides is the peptide His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 and other peptides which cause the release of growth hormone by essentially the same mechanism as His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2. Another preferred growth peptide is Ala-His-D-Nal-Ala-Trp-D-Phe-Lys-NH_2. Growth hormone releasing peptides are disclosed, for instance, in Momany, U.S. Patent 4,411,890; Momany, U.S. Patent, 4,410,513; Momany, U.S. Patent 4,410,512; Momany, U.S. Patent 4,228,158; Momany, U.S. Patent 4,228,157; Momany U.S. Patent 4,228,156; Momany, U.S. Patent 4,228,155; Momany, U.S. Patent 4,226,857; Momany U.S. Patent 4,224,316, Momany U.S. Patent 4,223,021; Momany, U.S. Patent 4,223,020; Momany, U.S. Patent 4,223,019; Bowers et al., U.S Patent 4,880,778; Bowers et al., U.S. Patent 4,880,777; Bowers et al., U.S. Patent 4,839,344; Bowers et al., U.S. Patent WO 89/10933 (PCT/US89/01829); Bowers et al., EP-A-398 961, Bowers et al. EP-A-400 051.

[0047] The pharmaceutically-active compounds employed in the present invention also include immunogens which can be incorporated into vaccine adjuvant systems. The immunogens which are acceptable include purified proteins and peptides and derivatives thereof, and generally immunogens which have a weight average particle size in the range up to 150 nm which therefore are capable of being maintained in the aqueous phase of the microemulsion.

[0048] The biologically active material is said to be a "water-soluble" material. Those skilled in the art will readily understand by the list of representative active materials that they are soluble to an effective extent in an aqueous phase and have negligible solubility in an organic phase. The solubility of the active materials in the aqueous phase at 20°C is at least 1 part per 100,000 parts and preferably at least 1 part per 10,000 parts. To achieve this level of solubility the pH or ionic strength of the aqueous phase may be altered. The solubility of the active materials in organic materials, such as those stated comprising the organic phase of the microemulsion, at 20°C is loss than 10 parts per 1,000,000 parts and preferably less than 1 part per 1,000,000 parts. The water:oil partition coefficient is greater than 10:1, advantageously at least 50:1, preferably at least 100:1, and most preferably greater than 1000:1. The water:oil partition coefficient is a commonly used quantity and refers to the ratio of the solubility of the material in water at 20°C to the solubility of the material in a reference oil, generally olive oil which is a mixture of trigylcerides of saturated and unsaturated fatty acids esterified to gylcerol, at 20°C. The partition coefficient is determined by dissolving the active agent in an equal volume of water and olive oil (absent surfactant) and determining the solubility in each phase. As used herein, the reference oil is a U.S.P./N.F. grade olive oil available from various chemical suppliers including Spectrum Chemicals Mfg. Corp., Gardena, CA.

[0049] The amount of active ingredient included in the internal aqueous phase may be varied considerably, depending upon its solubility and activity, the use for which it is intended, the amount of emulsion to be employed, and the like. Generally, as stated above, active ingredients in the amounts of 10^{-9} to 100% by weight/volume %, based on the volume of the internal aqueous phase, provide a satisfactory formulation for most applications. The biologically active material will either be soluble in the w/o microemulsion or it will be soluble upon the conversion to the o/w emulsion upon the addition of water to the system.

[0050] The w/o microemulsions may be formulated with agents for enhancing mucosal absorption of peptides and proteins. These include bile salts such as trihydroxy bile salts, i.e. cholate, taurocholate, and glycocholate, dihydroxy bile salts, i.e. deoxycholate, taurodeoxycholate, chenodeoxycholate, and ursodeoxycholate, triketo bile salts such as dehydrocholate. Non-ionic surfactants such as polyoxyethylene ethers with alkyl chain lengths from 12-18 carbon atoms and polyoxyethylene (POE) chain lengths from 2-60, p-t-octylphenoxypolyoxyethylenes with 2-60 POE groups, nonylphenoxypolyoxyethylenes with 2-60 POE groups, polyoxyethylene sorbitan esters with 8-24 alkyl chain lengths and 4-80 POE groups, and 1-dodecylhexahydro-2H-azepin-2-one(azone, laurocapram) can be used. Anionic surfactants such as sodium dodecyl sulfate and dioctyl sodium sulfosuccinate can be used. Lysolecithins containing saturated fatty acyl chains having 8-24 carbon atoms or unsaturated fatty acyl chains having 1 to 4 double bonds and 16-24 carbon atoms can be used. Mono/diesters of glycerol, such as medium chain fatty acid mono/di-esters containing saturated fatty acids with 8-12 carbon atoms, and mono/di-glycerol esters of unsaturated fatty acids having 1 to 4 double bonds and 16-24 carbon atoms can be used. Acylcarnitines, acylcholines and acylamino acids can be used, such as acylcarnitines having 12-20 carbon acyl groups and where the acyl groups have 0-4 double bonds, acylcholines such as acyl choline esters of fatty acids having 8-22 carbon atoms and 0-4 double bonds, and acylamino acids such as N-acyl amino acids and dipeptides having acyl groups with 8-24 carbon atoms and 0-4 double bonds and the amino acids having α or β amino groups and a molecular weight loss than 350. Additionally, mono and polyunsaturated fatty acids and their salts having 14-24 carbon atoms and 1-4 double bonds, and salicyclic acid and its sodium salt, sodium 5-methoxy-salicylate can be used.

[0051] The w/o microemulsions of this invention may readily be prepared by simply mixing together with mild agitation the selected components in the desired ratios at room temperature or at slightly elevated temperatures. As pointed out above, no high-energy mixing or application of heat is necessary, although limited use of each may be employed, if desired, to increase the rate of formation of the microemulsion. Moreover, the ingredients do not have to be added in any particular order other than that the active material be present in the aqueous phase as the emulsion is formed. Preferably, however, the surfactant should first be mixed with the oil phase, followed by the addition of water in the proper ratio. It is preferred to dissolve the active material in the water first, and then add this aqueous phase to the oil and sur-

factant components.

[0052] The size of the droplets, i.e., the number average diameter, in the resulting w/o microemulsion is usually 10-150 nanometers (nm), usually below 50-100 nm, with the majority of droplets below 100 nm, more preferably below 75. The particle size measurement is usually determined by laser light scattering techniques. The water-in-oil microemulsions are also characterized by their stable, clear homogeneous appearance.

[0053] The amount of water or aqueous fluid, e.g. aqueous body fluid, necessary to convert the w/o emulsion to an o/w emulsion when used, for example, for storing proteins, is not critical and may be determined routinely by titration of the microemulsion with excess water. Generally, however, it has been found that water in excess of 1 to 33 times that of the volume of the emulsion is sufficient for this purpose.

[0054] Besides the volume of water added or provided by the body itself, other factors which control the rate of release of any given drug include pH, temperature, and degree of agitation. Those skilled in the art will recognize that by varying these conditions in a generally known manner, the release of the drug can be slowed or increased as desired.

[0055] The microemulsion system of the present invention can be formulated with a high melting oil, that is, an oil with a melting point above room temperature (22-23°C), preferably above 30°C, in order to formulate a microemulsion which is a solid at room temperature. Also, high melting surfactants such as a C_{10-40} ester of a long chain fatty acid and alcohols having at least 12 carbon atoms, wherein these surfactants have melting points above room temperature, preferably above 30°C. Preferably, the microemulsion will melt at body temperatures, generally between 35-40°C. The amount of high melting oil and the melting point of that oil can vary, but the final composition containing the microemulsion is solid at room temperatures. The solid microemulsion system can be used as a suppository transport vehicle or as an oral transport vehicle. The oral formulation is preferably in tablet or capsule form. The microemulsion can either be formulated directly with the high melting oil, or the microemulsion can be formulated first, after which the high melting oil is blended with the microemulsion. Such high melting oils are well known in the art and include, for example, partially hydrogenated coconut oils, palm oils, cocobutter, hydrogenated peanut oil, and various hydrogenated vegetable oils, along with combinations thereof. Preferred oils include hydrogenated coconut and palm oils and mixtures thereof.

[0056] The w/o microemulsion system that is solid at room temperature (22-23°C) can be prepared using the high melting oil directly with the other components during formulation. The solution of components is heated to a slightly elevated temperature of from 25-60°C, preferably 30-50°C, during mixing and cooled to a solid at room temperature. The final w/o microemulsion system has component ranges within those previously stated for the liquid microemulsion systems. Preferred solid systems have from 20-90%, preferably 30-60% w/w of a high melting oil having a melting point from 85-120°F; from 1-50%, preferably 3-30% w/w of the aqueous phase, and 15-80%, preferably 23-60% w/w of a surfactant or surfactant mixture having an HLB range as set forth in this invention. Preferably, the surfactant is a mixture of surfactants containing 5-30%, preferably 8-20% w/w (of the microemulsion) of a surfactant having an HLB greater than 8, and 10-50%, preferably 15-40% w/w (of the microemulsion) of a surfactant having an HLB lower than 8.

[0057] The w/o microemulsion system that is solid at room temperature can also be prepared by first preparing the w/o microemulsion without the high melting oil and dispersing this microemulsion in the high melting oil. First, the w/o microemulsion is prepared according to the present invention. Then, the high melting oil is blended with the w/o microemulsion. Commonly this is accomplished at slightly elevated temperatures between 25-60°C, preferably 30-50°C. The microemulsion is thereby dispersed within a matrix made of the high melting oil. The amount of high melting oil to microemulsion ranges from 0.5:1 to 2:1. This amount can vary beyond these ranges so long as a final dispersed microemulsion system is produced which is a solid at room temperature. The high melting oil is typically admixed with a low HLB surfactant, generally having a HLB below 8, prior to addition to the microemulsion in order to properly retain and disperse the microemulsion in the high melting oil.

[0058] It has been surprisingly found that by taking a certain w/o microemulsion system of the present invention, and adjusting it to have a higher effective HLB value, that the w/o microemulsion converts, upon addition of water, not just to an o/w emulsion as do all of the claimed w/o microemulsions, but rather to an o/w microemulsion. The higher HLB value is obtained in the present systems by the addition of a modifier which allows the w/o microemulsion HLB level to be increased beyond its normal stability level without the breaking of the w/o microemulsion. The final HLB level of the surfactant or surfactant mixture of these w/o microemulsions is greater than 7, and is preferably from 7 to 16, most preferably from 8-13. Modifiers found to be useful are incorporated into the aqueous phase of the microemulsion and include sorbitol, polyethylene glycol (PEG), mannitol, propylene glycol, and mono- and disaccharides. If proteins or peptides are incorporated into the aqueous phase, then preferred modifiers are mannitol, sorbitol, and PEG.

[0059] The more modifier added to the w/o microemulsion, the higher the HLB can be raised in the system with the retention of a w/o microemulsion. This higher HLB level allows for conversion to an o/w microemulsion. The precise amount of modifier and the precise amount of higher level HLB surfactant added to the w/o microemulsion is functionally determined by the presence of two end results: (1) the retention of the w/o microemulsion and (2) the conversion to an o/w microemulsion upon addition of water.

[0060] The amount of modifier added to the aqueous phase of the w/o microemulsion depends on the desired final HLB. Typically, a 10-50%, preferably a 20-50%, most preferably a 20-30% by weight aqueous modifier solution, prefer-

ably a sorbitol solution, can be employed as the modified aqueous phase for the w/o microemulsion. This sorbitol solution can contain physiological buffers and saline or other salts.

[0061] The particle size of the w/o microemulsion which converts to an o/w microemulsion is the same as aforestated for the w/o microemulsions. The number average particle size of the converted o/w microemulsion is typically below 100 nm, preferably between 10-100 nm, most preferably between 20-60 nm as determined by laser light scattering technique. The amount of water required to convert the w/o system to the o/w microemulsion can vary depending upon the composition of the w/o microemulsion. Typically the amount of water required ranges from 1 to 10 times the volume of the w/o system. Larger amounts of water can be used to convert the w/o systems, and amounts up to 1000 times the volume of the w/o system, preferably 3 to 100 times the volume of the w/o system are used to convert to the o/w microemulsion.

[0062] These w/o converting to o/w microemulsion systems can be advantageously employed as transport vehicles for water soluble drugs which degrade in the oil phase, such as certain peptides, proteins, and immunogens used for oral or suppository formulations. Also, these formulations are preferred for intravenous and intraarterial administration. The risk of emboli formation is greatly reduced due to the exceedingly small particle sizes produced upon conversion with excess bodily fluid.

[0063] These w/o converting to o/w microemulsion formulations can also be used as nutritional lipid emulsions, and especially as total parenteral nutrition formulations. The w/o system can be converted using an aqueous phase containing water soluble nutrients to form lipid-in-water microemulsions just prior to administration.

[0064] The w/o microemulsions containing the biologically active material in the aqueous phase of the present invention are preferably administered parenterally, enterally and via other mucous membranes such as nasally, rectally, vaginally, or via the colon. After administration, the biological effect upon the animal caused by the active material can be measured or observed. The convertible microemulsion system enhances both the drug activation and uptake at the site of conversion. The unique convertibility feature of the present microemulsions provides that the drug will be maintained primarily in the aqueous phase due to oil phase insolubility. This is advantageous in that certain active materials may become inactivated if dispersed within an oil phase or if dissolved within an aqueous phase outside of an emulsion. Generally, such active materials as proteins and peptides employed in the present invention display a greater activity level when stored in the o/w microemulsion system as compared to their being stored for the same period of time and under the same conditions in the same aqueous phase that is not contained within an emulsion system.

[0065] The oral administration of a biologically active material, contained within the w/o microemulsion drug delivery system of the present invention, can be in the form of a capsule or tablet. The capsule is generally a starch or gelatin material. Certain active materials may be susceptible to the low pH environment of the stomach and should therefore be delivered to the higher pH environment of the intestinal system. Although such active materials are beneficially delivered in suppository form, if oral delivery is desired, the capsule or tablet can be supplied with an enteric coating. Such coatings are well known in the art as are the methods of enterically coating a capsule or tablet. The method of producing an enterically coated capsule using the w/o microemulsion system of the present invention is as follows. The w/o microemulsion containing the active agent is prepared and this composition is then placed into a capsule. The capsule is then coated with an enteric coating solution. The enteric coating solution contains the polymeric enteric coating substance and solvents. The polymeric enteric coating substance is generally a pharmaceutically acceptable polymer that will dissolve upon contact with intestinal fluids, pH of 5.5 to 7.0, but will not dissolve in the lower pH stomach fluids. Enteric polymer coatings are readily available commercially, such as the Eastman® C-A-P™ (cellulose acetate phthalate) and C-A-T (cellulose acetate trimellitate) enteric coating materials available from Eastman Chemical Products, Inc. Various techniques are known to apply the entire polymer coating such as spray coating or immersion coating and several layers of the enteric substance may be required.

[0066] A preferred w/o microemulsion system for the delivery of a biologically active material, such as calcitonin, to the gastrointestinal tract is one which is both a solid at ambient conditions and which converts into an o/w microemulsion upon contact with an aqueous medium such as bodily fluids. An example of such a preferred system is one containing 33-45% v/v, most preferably 36-42%, of a composition containing a mix of triesters:diesters of glycerol and lauric acid having a melting point of 33-36°C (an example being Witepsol H-15 which is a 90:10% wt. mixture of triesters:diesters with a small, less than 2% wt., amount of monoglycerides made by Huls of Germany); 30-42% v/v, most preferably 32-40%, of polyoxyethylene sorbitan monooleate (Tween® 80, Sigma Corp.); 5-10% v/v, most preferably 6-9%, of mono-/di-glycerides of medium chain fatty acids, capric and caprylic (Capmul MCM, from Karlshamns Lipid Specialties, Columbus, OH); 3.5-5.5% v/v, most preferably 4-5% of a long chain monoglyceride, such as sunflower oil monogylcerides (Myverol® 18-92); and 3-25% v/v, most preferably 5-20%, of an aqueous 20% w/v sorbitol in buffer solution containing the biologically active material. The drug content, pH, and ionic strength of the aqueous solution will vary depending on the composition that is most suitable for the hosted biological active material. If calcitonin is used, it is preferred to employ up to about 1 mg of salmon calcitonin (from Bachem Co.) per gram of the microemulsion system.

[0067] A preferred w/o microemulsion system for the delivery of a biologically active material, such as calcitonin, in a suppository form is one which is a solid at room temperature. An example of such a preferred system is one containing

23-27% w/w propylene glycol esters of capric/caprylic acids (Captex® 200 from Karlshamas Lipid Specialties, Columbus, OH); 6-10% w/w mono- and diglycerides of caprydic/capric acids (Capmul 8210 MCM from Karlshamas Lipid Specialties); 1-2.5% w/w liquid lecithin from Central Soya (Centrophase® 31); 15-17% w/w polyoxyethylene glycerol triricinoleate (Cremophor® EL from BASF); 40-45% w/w partially hydrogenated palm kernel, coconut and palm oils (HB-108 from Karlshamas Lipid Specialties), and 5-7% w/w 100 mM acetate buffer, p.H.=4.2. When used in a calcitonin suppository, it is preferred to use about 980U salmon calcitonin (from Bachem Co.) wherein the weight of the final suppository is about 1.7g.

[0068] Another preferred system for delivery of the active material is a composition containing from 5-80% v/v of a mixture of triesters:diesters of glycerol and lauric acid having a melting point of 33-36°C (an example being Witepsol® H-15); 15-50% v/v of polyoxyethylene sorbitan monooleate (Tween® 80); 3-11% v/v of mono-/di-glycerides of medium chain fatty acids, capric and caprylic (Capmul® MCM); 2-6% v/v of a long chain monoglyceride, such as sunflower oil monogylcerides (Myverol® 18-92); and 6-42% v/v of an aqueous 25% w/w sorbitol and 25% w/w propylene glycol in buffer solution containing the biologically active material. The drug content, pH, and ionic strength of the aqueous solution will vary depending on the composition that is most suitable for the hosted biological active material. This composition is preferred for the administration of such active agents as calcitonins, insulins, human growth hormones, fibrinogen receptor antagonists (RGD containing peptides, such as cyclo(S,S)-N^{α}-acetyl-Cys-(N^{α}-methyl)Arg-Gly-Asp-Pen-NH_2), and growth hormone releasing peptides, such as His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2.

[0069] As aforestated, in yet another embodiment, our microemulsions may be used to prepare non-drying topical, as opposed to transdermal, salves and ointments. These may readily be prepared by simply admixing a therapeutically-active amount of the emulsion with known topical petroleum bases or the like customarily employed for skin application, as long as these materials are compatible with the emulsion. The w/o microemulsion is ideally suited for wound care treatment where the dry epidermal skin layer, the stratum corneum or horny layer, is removed thereby exposing the aqueous-based dermal skin layer, as for example in burn wounds. The w/o microemulsion can also be used where the dermal skin layer is also partially removed. The w/o microemulsion, when contacted with the dermal or lower body layer converts to an o/w emulsion upon the addition of aqueous bodily fluids. Preferably, proteases, such as serine, metallo, cysteine, aspartyl, and the like which degrade connective tissue proteins such as collagen and elastin and the like, along with growth factors are used as the active material to aid in the removal and repair of skin tissue. Examples of growth factors include, for example, platelet derived growth factor, PDGF, epidermal growth factor, EGF, transforming growth factors, TGFα and TGFβ, and insulin-like growth factor, IGF-I and IGF-II, and the like. These active materials generally have average particle sizes of greater 1 to 100, preferably from 3 to 30, nanometers. Typically, the molecular weight of these active materials is at least 5000 and up to over 40,000, preferably from 5,000 to 35,000. The average human epidermis pore size is below 1 nm, and therefore the active materials employed in the topical systems do not effectively traverse the epidermis skin layer.

[0070] The topical microemulsion system acts as a resevoir for providing a stable protein to the wound site. The topical microemulsion is preferably presented in the form of a solid, salve, or gel that can be easily removed from the wound site by washing with aqueous fluid. Most preferably, the topical is presented as a solid or semisolid (deforming upon application of pressure) to maintain the w/o microemulsion at the wound site for conversion and release of the drug.

[0071] A further embodiment of the present invention encompasses the use of the w/o microemulsion as a carrier system to be used in a vaccine adjuvant system. In such a vaccine adjuvant system, the immunogen is admixed into the aqueous phase. This aqueous phase is then admixed with the oil phase which contains the surfactant. These adjuvant systems can also be formulated with an immuno-stimulator which are well-known in the vaccine adjuvant art. Such immuno-stimulators include such compounds as muramyl di-or tri-peptide and derivatives thereof; interferons, and interleukins. The aqueous phase may also contain inorganic salts, buffering agents, preservatives, and the like, in addition to the immunogen.

[0072] The microemulsion vaccine adjuvant system of the present invention is characterized by its stability and long shelf life, in comparison to emulsion adjuvant systems of the prior art. The use of the oils of the present invention, which are referred to as biodegradable oils, to formulate the microemulsion system provides benefits over previous emulsion adjuvant systems in that the production of granulomas is believed to be decreased. The w/o microemulsion adjuvants can readily convert to oil-in-water emulsions when administered into the body which allows for the generation of macrophage stimulating oil droplets *in situ*. The smaller and more uniform size of the resulting droplets also is expected to lead to a more reproducible response to a given immunogen.

[0073] The invention will now be illustrated by, but is not intended to be limited to, the following examples.

EXAMPLES

Formulation and Convertibility

[0074] Several formulations of the water-in-oil (w/o) microemulsions of this invention were prepared in which, by way

of illustration, the components, their ratios, and the operating conditions selected to provide a convertible microemulsion, were varied somewhat as shown in the following examples. For convenience, a drug was not included in every instance, but it will be understood that any water-soluble drug, as defined above and as shown in some of the Examples, would be dissolved in the dispersed water phase.

[0075] The HLB value of each surfactant system and stability of each emulsion was then determined, as set forth below in each example.

[0076] For the purposes of these examples, the HLB values used were those specified by the suppliers of the surfactants; the resulting HLB of a mixture of surfactants was calculated on a volume basis.

[0077] In preparing each formulation, the following general procedure was employed:

[0078] Into a small vial was pipetted a measured amount of oil, followed by the addition of a surfactant, or mixture of surfactants, of a given HLB value. The vial was then shaken with a vortex mixer for a given number of minutes until the surfactant and oil were evenly mixed. A saline solution was then added to the oil/surfactant mixture and the mixture shaken a few minutes until an optically clear w/o emulsion was recovered. Its stability is measured by periodic visual inspection for the presence of macroscopic phase separation, as shown by cloudiness or the formation of two distinct layers. Stable means the emulsion is clear and single phase.

[0079] The physical characteristics of the microemulsions can be tested including such properties as viscosity, conductance and refractive indices.

EXAMPLE 1

[0080] In accordance with the foregoing general procedure, a w/o microemulsion was prepared employing the following components, amounts and ratios, and HLB values of the surfactants:

Component	Composition	HLB Value	Amount (µL)
Oil	Captex®200[1]		870.0
Surfactant System	POE 50 Sorbitol Hexaoleate[2]	11.4	50.0
	Cremophor EL	13.5	50.0
Water	Saline (0.9wt.% NaCl)		30.0
TOTAL		12.5	1000.0

[1] Captex®200 - propylene glycol esters of capric/caprylic acids (Karlshamns Lipid Specialties, Columbus, OH)

TABLE 1

Physical and Chemical Characteristics of Captex 200

Description:	Diester manufactured by reesterification of fractionated coconut fatty acids (primarily caprylic and caproic) with propylene glycol.
CTFA Name:	Propylene glycol dicaprylate/caprate
Free Fatty Acid (As Oleic):	0.03
Hydroxyl Number:	0.05
Saponification Number:	329.7

Fatty Acid Composition:

Caproic	4.1
Caprylic	68.2
Capric	27.4
Layric and higher	0.2

[2] POE Sorbitol Hexaoleate - polyoxyethylene (50) sorbitol hexaoleate (ICI Americas, Inc. Wilmington, DE)

[3] Cremophor® EL - Polyoxyethylenglycerol Triricinoleate 35 DAC (BASF, Inc.)

[0081] These components were mixed in a vortex mixer at 25°C for about 3 minutes to provide a clear stable w/o microemulsion.

[0082] Water was then added to the total composition in the ratio of 4:1 (v/v) to convert the microemulsion to an o/w emulsion.

EXAMPLE 2

[0083] In accordance with the procedures of Example 1, the following components were employed to form a w/o microemulsion:

Component	Composition	HLB Value	Amount (μL)
Oil	Captex® 200		870.0
Surfactant System	Centrophase® 31*	4.0	10.5
	Cremophor® EL	13.5	89.5
Water	Saline (0.9 wt.% NaCl)		30.0
TOTAL		12.5	1000.0

* Centrophase® 31 - lecithin (mol. wt. - 800) (Central Soya, Fort Wayne, IN).

[0084] These components were mixed in a vortex mixer at 25°C for about 6 minutes to provide a clear w/o microemulsion which was stable, at both 25°C and 50°C.

[0085] Water was then added to the total composition in the ratio of 4:1 (v/v) to convert the microemulsion to an o/w emulsion.

EXAMPLE 3

[0086] In accordance with the procedures of Example 2, but substituting 54.5 μL of Tween® 80 (polyoxyethylene-sorbitan monooleate, Sigma Corp.) (HLB = 15) for Cremophor® EL, and increasing the amount of Centrophase® 31 to 45.5 μL to provide an average HLB value of 10.0, a w/o microemulsion was formed and converted to an o/w emulsion.

EXAMPLE 4

[0087] In accordance with the procedures of Example 1, the following components were employed to form a w/o microemulsion:

Component	Composition	HLB Value	Amount (μL)
Oil	Captex 200		861.3
Surfactant System	Capmul MCM*	5.0	8.7
	Centrophase 31	4.0	10.5
	Cremophor EL	13.5	89.5
water	Saline (0.9 wt.% NaCl)		30.0
TOTAL		9.0	1000.0

* Capmul MCM - mono - and diglycerides of medium-chain fatty acids (capric and caprylic) (Karlshamns Lipid Specialties, Columbus, OH).

[0088] These components were mixed in a vortex mixer at 25°C for about 3 minutes to provide a clear w/o microemulsion having a particle size of 25 nm (number average) and a stability from 5°C to 50°C as measured by periodic visual inspection.

[0089] Water was then added to the total composition in the ratio of 4:1 (v/v) to convert the microemulsion and produce o/w emulsion.

EXAMPLE 5

[0090] In accordance with the procedures of Example 2, but increasing the amount of water (saline) from 30 to 150 μL to provide 15% water in the formulation, and adjusting the amounts of the other components accordingly (oil - 350 μL; Centrophase 31 - 52.6 μL; Cremophor EL - 447.4 μL), the w/o microemulsion satisfactorily converted to an o/w emulsion. In this formulation, the ratio of oil-to-water was 2.3:1, and that of surfactant-to-water plus oil was 1:1.

EXAMPLE 6

[0091] In accordance with the procedures of Example 4, but altering the amount of Capmul surfactant, first to 4.35 µL (final HLB = 10.2), and then to 17.4 µL (final HLB = 7.7), convertible microemulsions were also obtained.

EXAMPLE 7

[0092] In accordance with the procedures of Example 4, but substituting 8.7 µL of 1-monocapryloyl-rac-glycerol, or 8.7 µL of Dicaprin (an equimolar mixture of 1,2- and 1,3-diglyceride of Capric acid), for the Capmul® MCM surfactant, satisfactory convertible microemulsions were also obtained.

EXAMPLE 8

[0093] In accordance with the procedures of Example 2, but substituting Myverol® 18-92 (glycerol monolinoleate; HLB value - 3.8-4.0) for the Centrophase® 31 surfactant of that surfactant system, and mixing the components for 3 minutes, there was obtained a w/o microemulsion which, when water was added (4:1 v/v), converted to an o/w emulsion. The HLB of the surfactant mixture in this formulation was 9.0.

EXAMPLE 9

[0094] In accordance with the procedures of Example 4, but substituting 861.3 µL of Myvacet® (1-oleyl-2,3-diacetyl glycerol); (Eastman Chemical Products, Inc., Kingsport, TN) for the Captex® 200 as the oil, there was obtained a satisfactory w/o microemulsion which, upon addition of water to the total composition (in the ratio of 4:1 v/v), converted to an o/w emulsion. The HLB of the surfactant mixture in this formulation was 9.0.

Stability Data

[0095] In order to demonstrate the stability of the compositions of this invention at elevated temperatures for purposes of storing the same for long periods of time, a series of microemulsions was prepared in accordance with this invention, following the general procedures of Example 2. In Example 10, the protein horseradish peroxidase (HRP), was stored for given times and temperatures, then assayed *in vitro*, as shown in this example.

EXAMPLE 10

[0096] This example illustrates the incorporation of a protein, namely the enzyme horseradish peroxidase (HRP), in the convertible w/o microemulsion of this invention, and the stability of this resulting emulsion.

[0097] In accordance with the general procedures above, an enzyme-containing microemulsion was prepared front the following components:

Component	Composition	HLB Value	Amount (µL)
Oil	Captex® 200		861.3
Surfactant System	Capmul® MCM	5.0	8.7
	Centrophase® 31	4.0	10.5
	Cremophor® EL	13.5	89.5
Peroxidase Solution	(see Footnote 1)		30.0
TOTAL		9.0	1000.0

1 Peroxidase solution - 100 µL of HRP stock solution (1 mg/mL) in 400 µL of 0.9 wt.% saline (NaCl) solution.

[0098] These components were mixed in a vortex mixer at 25°C for about 2 minutes to provide a w/o microemulsion.

[0099] After storage for the specified time at 50°C, the microemulsion was then converted to an o/w emulsion by the addition of water. This was achieved by pipetting 30 µL of the microemulsion containing the horseradish peroxidase

enzyme into 970 µL of 0.9 wt.% saline (NaCl) solution.

[0100] After conversion, the emulsion was then assayed for activity. This activity was compared with the activity of stock solutions of HRP which had been maintained at 50°C for the same time and then pipetted into saline (30 µL into 970 µL of saline) in the same manner as the microemulsion above. The stock HRP was first diluted to the same HRP concentration as in the aqueous phase of the converted microemulsion.

A. Assay Procedure

[0101] The assay was carried out as follows:

1. Set spectrophotometer at 492 nm and 25°C.
2. Into the cuvette, pipet 2.97 mL OPD (O-phenylene diamine) buffer solution (1 tab. - 26 mL)
3. Establish blank at 492 nm.
4. Into the cuvette, pipet 25 µL diluted control HRP solution. Mix and record the increase in absorbance at 492 nm for 5 minutes.
5. Same procedure is followed for microemulsion w/HRP solution. OPD = O-phenylene diamine

B. Results:

[0102] Percent activity was determined by using the following equation:

$$\text{Percent Activity} = \frac{\text{Activity at time t}}{\text{Activity at time 0}} \times 100$$

[0103] The following table summarizes the results that were obtained from the assay for both Control HRP and microemulsion containing HRP.

TABLE 2

PERCENT ACTIVITIES OF BOTH CONTROL HRP (STOCK SOLUTIONS) AND MICROEMULSION CONTAINING HRP		
Time (Hours)	**% Activity**	
	Control HRP	**HRP in ME**
0	100	100
3	76	77
6	73	83
24	20	68
27	20	68
48	11	53

[0104] From the foregoing results, it will be seen that after 48 hours, the microemulsion containing HRP was much more active than the control HRP, which had lost most of its activity by 48 hours. Thus, the microemulsion of this invention provides the distinct advantage of permitting long-term storage of proteins at elevated temperatures, whereas heretofore they had to be maintained at much colder temperatures to preserve their stability.

EXAMPLE 11

[0105] A series of experiments was carried out in rats using the w/o microemulsions of this invention to evaluate them as a vehicle for the rectal delivery of the peptide calcitonin, (used in the treatment of hypercalcemia by lowering Ca++

serum levels), whereby the body fluids of the rat would serve to convert the microemulsion to an o/w emulsion and thus release the calcitonin.

[0106] Formulations were produced which ranged from 3% to 15% (v/v) aqueous phase and which ranged from liquids to gels at room temperature. The formulations contained, in addition to the aqueous phase, one to three oils and a blend of two emulsifiers. Most formulations showed temperature stability over the range from 5°C to 50°C. Three formulations with different oil blends were chosen for biological evaluation in juvenile, male rat model (Sprague-Dawley rats; 140-170 gm).

[0107] Rectal installation was compared with direct injections of calcitonin into the body. As shown by the data below, rectal instillation of each of the three microemulsion calcitonin formulations tested produced a dose dependent lowering of serum calcium in the rat, thereby demonstrating that the w/o microemulsion had been converted in the colon, with the release of effective amounts of active calcitonin. Control microemulsion preparations, on the other hand, which did not contain calcitonin, did not produce a significant change in serum calcium levels. Moreover, as shown below, incorporation of two oils plus coconut oil into the suppository to form a semi-solid microemulsion improved the calcitonin response by more than tenfold over the basic liquid formulation containing a single oil.

A. Formulations

[0108] Three w/o microemulsion formulations were tested which contained 3% v/v aqueous phase volume, and varying amounts of calcitonin/ml of emulsion. Two, Formulations A and B below, were formulated as liquids; the third microemulsion (Formulation C) was formulated in semi-solid (suppository) form by addition of a high-melting coconut oil to the microemulsion. This formulation was a soft waxy solid at room temperature which melted at body temperature to release calcitonin via the microemulsion.

Key to Calcitonin Microemulsions Formulations:

[0109]

A. The microemulsion of Example 2, plus calcitonin.
B. The microemulsion of Example 4, plus calcitonin.
C. The microemulsion of Example 4, (1 volume,); to which is added 2 volumes of a mixture containing 1.8 volumes coconut oil and 0.2 volume Capmul MCM; plus calcitonin.

[0110] All calcitonin concentrations are given in units of biological activity per volume of final emulsion.

B. Test Methods

[0111] The calcitonin-containing, or just saline-containing, (control) microemulsions were administered rectally to each of a group of 3 to 7 rats in a volume of 250 µL. Blood samples were taken at time = 0, 1, and 2 hours after dosing. Serum calcium was measured after 1 and 2 hours because initial studies showed that this is when maximal calcitonin response was obtained. The rats were anaesthetized throughout the entire procedure and were bled via the orbital sinus.

[0112] Serum was prepared from each blood sample and serum Ca^{+2} (free ionized calcium) levels were determined using a Beckman calcium clinical assay kit.

C. Results

[0113] The results of this study are shown in Table 2 which summarizes the activity of Microemulsions A, B and C.

TABLE 3

EFFECT OF RECTALLY-INSTILLED CALCITONIN MICROEMULSIONS ON SERUM CALCIUM LEVELS				
Microemulsion	Calcitonin Content (units/ml)	No. of Animals	Change in Serum Ca^{+2} at 1 Hr. after Treatment (mg/dL)±SD[1]	After 2 Hrs.[1]
A	0	4	0.23 ± 2.55	1.92 ± 1.01
	60	7	-1.81 ± 2.50	-1.02 ± 1.65
	120	5	-1.11 ± 0.96	-1.60 ± 1.25
	240	5	-1.89 ± 1.27	-2.44 ± 1.29
B	0	4	-0.38 ± 1.58	0.73 ± 0.91
	10	4	-1.78 ± 0.78	-1.30 ± 0.50
	20	5	-1.98 ± 0.47	-2.36 ± 0.44
C	0	3	0.17 ± 0.09	0.67 ± 0.50
	10	4	-1.71 ± 0.51	-2.39 ± 0.36
	20	4	-1.82 ± 0.35	-2.23 ± 0.11
Preconverted B	0	5	0.41 ± 0.13	0.47 ± 0.40
	20	5	-1.27 ± 1.07	-1.62 ± 1.29
Saline	10	5	-0.13 ± 0.45	0.15 ± 0.33

[1] Ionized calcium in blood serum in units of milligrams of calcium (mg) per deciliter (100 mL) of serum ± the standard deviation.

[0114] The results shown in Table 2 show the effectiveness of our microemulsions containing calcitonin in lowering serum calcium. Because of the higher response of ME-B compared to ME-A, we needed to determine that the lower response of ME-A was not due to deactivation of the calcitonin by the formulation itself. To determine this, 250 µL of ME-A (60 Units/mL) and ME-A (0 Units/mL) were injected SQ into 2 pairs of animals. The serum calcium fell an average of 3.2 mg/dL (milligrams/deciliter) in the calcitonin microemulsion-treated animals, and 0.3 mg/dL in the controls. This demonstrates the presence of active calcitonin in ME-A.

[0115] Another series of tests were performed to demonstrate the efficacy of these emulsions which were converted after storage but before administration into the rats. In accordance with these tests, Microemulsion B was formulated and stored at 5°C for 2 days, following which it was converted to an o/w emulsion by addition of water equal in amount to that of the total volume of the emulsion prior to rectal introduction into rats. As shown in Table 2, the calcitonin was generally effective after storage when pre-converted and then used, but not as effective as internal conversion within the colon.

[0116] The table also shows that incorporation of the mono-and diglycerides surprisingly produced a significant improvement in the response to calcitonin. A dose of 20 U/mL of ME-B produced a response similar to that previously obtained at 240 U/mL of ME-A, more than an order of magnitude improvement.

[0117] Rectal administration of the solid calcitonin microemulsion C produced responses that were equal to or greater than those seen with the B formulation.

[0118] The last line of Table 2 indicates that instilling a saline solution of calcitonin into the rectum produced no significant response.

EXAMPLE 12

[0119] The following example demonstrates that a non-convertible microemulsion wherein the surfactant HLB was 4.0, which was not effective in the rectal delivery of calcitonin.

[0120] A microemulsion was formulated as follows, using the general procedure of Example 1:

COMPONENT	COMPOSITION	HLB VALUE	AMOUNT (μL)
Oil	Captex® 200		500
Surfactant	Centrophase® 31[1]	4.0	450
Water Plus Calcitonin	Buffered Solution[2]		50
Total		4.0	1000

[1] Liquid Soybean Lecithin
[2] Calcitonin amount = 240 units/mL

[0121] The resulting calcitonin-containing w/o microemulsion was introduced into the colons of rats in accordance with the general procedures of Example 11. A measurement of the ionized calcium in the blood showed no significant decrease for the microemulsion system when compared to a control formulation with no calcitonin.

EXAMPLE 13

[0122] The following example demonstrates the production of w/o microemulsion systems which have relatively high water concentrations. In accordance with the above mentioned general procedure, w/o microemulsions were prepared employing the following components, amounts and ratios (volumes below are in microliters):

	Surfactant			Oil		Aqueous Phase	
Example	Myverol® 18-92	Tween® 20	Centrolene® A	Captex® 200	Triacetin	1% NaCl Solut.	Water
1	270	230	--	100	--	400	--
2	250	200	50	100	--	400	--
3	240	180	80	50	50	--	400
4	260	160	80	50	50	--	400
5	260	160	80	50	50	--	500
6	260	160	80	50	50	--	600
7	260	160	80	50	50	--	720

[0123] Tween® 20 is a laurate ester of sorbitol having a HLB value of about 16.7 purchased from Spectrum, New Brunswick, NJ. Centrolene® A is a hydroxylated lecithin having a HLB value of about 9.5 manufactured by Central Soya, Fort Wayne, IN.

EXAMPLE 14

[0124] A series of experiments was carried out using rats with the w/o microemulsion of this invention that are solid at ambient conditions to evaluate them as a vehicle for the oral delivery of the peptide salmon calcitonin (used in the treatment of hypercalcemia by lowering Ca^{2-} and PO_4 serum levels). The body fluids of the rat served to convert the microemulsion to an o/w emulsion which activated the drug and promoted drug uptake by the animal. The monitored variables were Ca^{2-} and PO_4.

Formulations

[0125] The test preparations were prepared using a high melting point oil, in this case a mixture of hydrogenated coconut and palm oil. The oils used were obtained from Karlshamns Lipid Specialties, USA, of Columbus, Ohio. The

oils were labeled HB-95, HB-108, and HB-118 which corresponded to the trade names of HYDROKOTE® 95, 108, and 118. The oils had an approximate melting point of 35, 42 and 48°C (95, 108, and 118°F)respectively.

[0126] The A group microemulsions were prepared by first formulating the microemulsion and then admixing the HB-108 oil with the microemulsion. The microemulsion components were mixed in a container at an elevated temperature of about 40°C to which was added the calcitonin contained in the acetate buffer. Once the microemulsion was formed, the HB-108 component containing 10% Capmul was added.

[0127] The B and C group microemulsions were prepared by formulating the microemulsion directly with the HB oil.

	FORMULATIONS	
	Group A1	**Control (A1')**
Dose	**40 U/mL**	**0 U/mL**
10% Capmul® MCM in Captex® 200	570 uL	1.71 mL
Cremophor® EL	298 uL	894 uL
lecithin	35 uL	105 uL
100mm acetate buffer	92 uL	300 uL
calcitonin stock sol'n 10,000 U/ml	8 uL	----
Total ME	1.0 mL	3.0 mL
10% Capmul® in HB-108	1.0 mL	3.0 mL
Total volume	2.0 mL	6.0 mL
	Group B1	**Control (B1')**
Dose	**40 U/mL**	**0 U/ml**
Myverol® 18-92	373 uL	746 uL
Tween® 80	404 uL	808 uL
Capmul® MCM	124 uL	249 uL
HB-95	725 uL	1.45 mL
100mm acetate buffer	365 uL	746 uL
calcitonin stock sol'n 10,000 U/mL	8 uL	----
Total Volume	2.0 mL	4.0 mL
	Group C1	**Control (C1')**
Dose	**40 U/mL**	**0 U/ml**
Myverol® 18-92	373 uL	746 uL
Tween® 80	404 uL	808 uL
Capmul® MCM	124 uL	249 uL
HB-118	725 uL	1.45 mL
100mm acetate buffer	365 uL	746 uL
calcitonin stock sol'n 10,000 U/mL	8 uL	----
Total Volume	2.0 mL	4.0 mL

Test Method

[0128] Each test group contained five animals (juvenile male rats, Sprague-Dawley rats approx. 140-170 gm). Group A1, B1 and C1 received 250 uL of the respective microemulsion, 40U/ml calcitonin; the controls received 250 uL of the control microemulsion.

[0129] The animals were orally gavaged with melted microemulsion and then quickly anaesthetized and a blood sample was taken via the orbital sinus to establish a baseline (T_0). After 120 min., a second blood sample was taken. The Ca^{2+} and PO_4 levels were analyzed in both samples and compared to determine the activation and uptake of the drug. Serum Ca^{2+} (free ionized calcium) levels were determined using a Beckman 700 calcium clinical assay kit along with serum PO_4 levels.

Results

[0130] The results of this study are shown in the table below which summarizes the activity of microemulsions A1, B1, and C1 and the controls A1', B1', and C1'. All microemulsion calcitonin formulations showed statistically significant reductions in both Ca^{2+} and PO_4 serum levels, except that the C1 emulsion system did not show such activity for reduction of Ca^{2+}. The 'P' value is a statistical quantity that refers to the probability that the treatment and control values are equal. A 'P' value of 0.05 represents a one-in-twenty chance that the groups are equal. Therefore, 'P' values below 0.05 are considered statistically significant.

SUMMARY OF SERUM CALCIUM AND PHOSPHATE CHANGES INDUCED BY ORAL GAVAGE OF RATS WITH MICROEMULSIONS CONTAINING HIGH MELTING POINT TRIGLYCERIDES WITH OR WITHOUT CALCITONIN

Formulation	Triglyceride	Calcitonin MRC U/mL	Ca^{2+} Diff. mg/dL vs. 2Hr	'P' Value Calcitonin vs. Control	PO_4 Diff. mg/dL vs. 2Hr	'P'Value Calitonin vs. Control
A1	HB-108	40	-0.62	--	-2.8	
A1'	HB-108	0	-0.14	0.029	-0.8	0.010
B1	HB-95	40	-1.58	--	-2.6	
B1'	HB-95	0	0.82	0.036	-0.2	0.003
C1	HB-118	40	2.08	--	-2.6	
C1'	HB-118	0	0.08	0.880	0.0	0.005

'P' Values < 0.05 are considered significant

EXAMPLE 15

[0131] A series of experiments was carried our using rats with the w/o microemulsion of this invention to evaluate the performance between solid formulations and liquid formulations using the peptide salmon calcitonin (used in the treatment of hypercalcemia by lowering Ca^{2+} serum levels) via oral administration. The body fluids of the rat served to convert the microemulsion to an o/w emulsion which activated the drug and promoted drug uptake by the animal. The serum Ca^{2+} was monitored to evaluate the effectiveness of the microemulsion carrier system.

Formulations

[0132] The solid test preparations were prePared using a high melting point oil, in this case a mixture of hydrogenated coconut and palm oil, HB-108 (HYDROKOTE® 108) which had a melting point of 48°C (108°F).

[0133] The A and B group microemulsions (ME) were prepared as liquid microemulsions at room temperature. The A ME was the liquid control and did not contain calcitonin. The group B ME was the liquid calcitonin sample. The C and D ME were prepared as solids at room temperature by first formulating the microemulsion and then admixing the HB-108 oil with the microemulsion. The microemulsion components were mixed in a container at an elevated temperature of about 40°C to which was added the calcitonin contained in the acetate buffer. Once the microemulsion was formed, the HB-108 component containing 10% Capmul® was added. The C ME was the control solid ME and the D ME was the calcitonin sample.

	FORMULATIONS			
Dose	**Group A Control**	**Group B 40 U/mL Calcitonin**	**Group C* Control**	**Group D* 40 U/mL Calcitonin**
Capmul® MCM	157 uL	157 uL	57 uL	57 uL
Captex® 200	1.413mL	1.413 mL	513 uL	513 uL
Centrophase® 31 (lecithin)	35 uL	35 uL	35 uL	35 uL
Cremophor® EL	298 uL	298 uL	298 uL	298 uL
Saline	100 ul	92 uL	100 ul	92 uL
Salmon Calcitonin 10,000 U/mL	----	8 uL	----	8 uL
HB-108**	----	----	0.9 mL	0.9 mL
Capmul® MCM**	----	----	0.1 mL	0.1 mL
Total Volumes	2 mL	2 mL	2 mL	2 mL

* The suppository base contained small amounts of Methylparaben, Propylparaben and BHT.

** The C and D ME were prepared first and these suppository base components added thereto to formulate the final ME which were solid at room temperature.

Test Method

[0134] Each test group contained four animals (juvenile male rats, Sprague-Dawley rats approx. 110 gm). Groups B and D received 250 uL of the respective microemulsion, 10U/ml calcitonin; the controls received 250 uL of the control microemulsion.

[0135] The animals were orally gavaged with the liquid ME and melted solid ME and then quickly anaesthetized and a blood sample was taken via the orbital sinus to establish a baseline. After 120 min., a second blood sample was taken. The Ca^{2+} level was analyzed in both samples and compared to determine the activation and uptake of the drug. Serum Ca^{2+} (free ionized calcium) levels were determined using a Beckman 700 calcium clinical assay kit.

Results

[0136] The results of this study are shown in the table below which summarizes the activity of microemulsions A, B, C, and D. The serum Ca^{2+} level after 120 min. was found to be significantly reduced, when compared to the control, in only the solid microemulsion formulation, ME D. The serum Ca^{2+} level was not significantly reduced using the liquid calcitonin sample, ME B, when compared to the control.

SUMMARY OF SERUM CALCIUM LEVELS TWO HOURS AFTER GAVAGE WITH LIQUID OR MELTED SOLID MICROEMULSIONS WITH OR WITHOUT SALMON CALCITONIN IN THE AQUEOUS PHASE					
Group	Treatment	Calcitonin MRC U/mL	Serum Ca+2 2Hr Post-dose	SD	'P'Diff.
A	Liquid ME	0	13.9	2.75	----
B	Liquid ME	40	12.2	0.82	0.860
C	Solid ME	0	13.5	2.89	----
D	Solid ME	40	9.0	2.70	0.033
'P' Values < 0.05 are considered significant.					

EXAMPLE 16

[0137] Stable w/o microemulsion formulations were prepared which, upon conversion with additional water, form o/w microemulsions. The w/o microemulsions were formulated with a sorbitol in saline solution which allowed for the formation of the w/o microemulsion at higher HLB values than those required to form a w/o microemulsion without the presence of the sorbitol solution. The higher HLB value allows for the system to convert into an o/w microemulsion.

[0138] Sample w/o microemulsions which convert to o/w microemulsions were prepared according to the systems described below. The HB-95 component is a purified coconut and palm oil mixture manufactured by Karlshamns Lipid Specialties of Columbus, OH, having a melting point of 35°C (95°F). Myverol® 18-92 is a surfactant having a HLB = 4 and is manufactured by Eastman Chemicals. Capmul® MCM is a surfactant having an HLB = 5.5-6.0 and is manufactured by Karlshamns Lipid Specialties. Tween® 80 is a surfactant having an HLB = 15 and was purchased from Spectrum Chemicals. The sorbitol was dissolved in a saline solution of 0.15 M NaCl. The HLB was determined using a volume average. The temperature was the temperature at which the microemulsion was formed.

[0139] The number average particle size of the converted microemulsion ranged from about 20 to about 70 nanometers. The amount of water used to convert the w/o microemulsion to the o/w microemulsion ranged from about 10 to about 1000 times the amount of the original w/o microemulsion volume.

w/o Converting to o/w Microemulsion Formulations

Sample ID	HB- 95 (µl)	Captex® 200 (µl)	Myverol® 18- 92 (µl)	Capmul® MCM (µl)	Tween® 80 (µl)	20% Sorbitol in Saline (µl)	30% Sorbitol in Saline (µl)	HLB	Temp. (°C)
A	--	700	130	90	650	360	--	12.4	25
B	700	--	130	90	650	--	360	12.4	40
C	400	300	140	160	570	460	--	11.5	37
D	400	300	100	160	610	460	--	12.0	37
E	400	300	60	160	650	460	--	12.5	37

EXAMPLE 17

[0140] A series of experiments was carried out using rats with the w/o microemulsion of this invention to evaluate them as a vehicle for the delivery of the human growth hormone, hGH. The body fluids of the rats served to convert the microemulsion to an o/w emulsion which activated the drug and promoted drug uptake across the mucosal membrane of the rat colon.

Formulations

[0141] The test microemulsion systems were prepared as set forth below. Group A was a suppository formulation made with a microemulsion formulation of the present invention. The Group A microemulsion was made first as a liquid and then dispersed within a high melting oil. The other groups were buffer solutions and not microemulsions.

Group A	
Captex® 200 with 10%Capmul® MCM	1.14ml
Lecithin	0.07ml
Cremophor® EL	0.59ml
hGH in sterile H_2O	0.20ml
HB-108 with 10% Capmul MCM	2.00ml

[0142] Group A contained 0.096U hGH/ml. Group B was a 5mM $NaPO_4$ buffer solution at pH=7.8 with 0.096U hGH/ml. Group C was a 5mM $NaPO_4$ buffer solution at pH=7.8 with 0.024U hGH/ml. Group D contained no hGH and was a 5mM $NaPO_4$ buffer solution at pH=7.8.

Test Method

[0143] Test rats were divided into four groups: A, B, C, and D. Groups A, B, and C received the extracted growth hormone while group D was a control and did not receive the hormone. The rats were approximately 100 grams and were fasted for 24 hours prior to testing.

[0144] The dosage and group size is shown in the table below. The injected group, Group C, received the extracted hGH in a buffer solution at the human equivalent dose of 0.05 mg/kg body weight. The two rectal administration groups, Groups A and B, received ten times the human equivalent dose.

Group	ROUTE	(Vol/dosage form)	[Drug]/rat	No.
A	Rectal	250 ul/supp.	.024 Units	18
B	Rectal	250 ul/buffer	.024 Units	12
C	SQ	100 ul/buffer	.0024 Units	12
D	Control	0	0	2

[0145] The rats were anaesthetized just prior to dosing. Suppositories (Group A) and the buffer solution (Group B) administered rectally were sealed in the rectum by a plug and liquid cement. Group C animals were injected subcutaneously (SQ). After administering the dosage, serum hGH levels were determined at 30, 60, 120, 180, 240, and 300 minutes. Three animals from Group A were used per data point. Two animals from Groups B and C were used per data point. Two animals were used at 0 minutes for a baseline in the control group, Group D. The blood samples were taken from the orbital sinus. The blood was centrifuged and the serum assayed by hGH ELISA (Medix Lab, Foster City, CA) for quantitation of the extracted growth hormone.

Results

[0146] At ten times the human equivalent dose level, the suppository formulations (Group A) showed an equivalent bioavailability to the injected dose (Group C). The AUC (area under the curve) for both routes of administration was determined using the trapezoid rule (M. Gibaldi, *Biopharmaceutics and Clinical Pharmacokinetics*, Lea and Febiger, Philadelphia, PA, 1984, pp. 315-16). The AUC was approximately 24.5 ng-hr/ml for both the SQ injection and the suppository. The hGH in buffer that was administered rectally at the same dose as the suppository formulation showed no uptake of the drug. The bioavailability of the suppository formulation was about 10% as compared to an injected dose.

Time (min)	Injected hGH (Group C)		Suppository hGH (Group A)	
	(ng/mL)	SD	(ng/mL)	SD
30	16.5	5.00	16.000	4.360
60	10.0	0.00	14.700	8.330
120	6.0	1.40	2.000	2.000
180	2.5	2.12	1.670	1.160
240	0.5	0.71	1.670	0.580
300	0.0	0.00	0.333	0.577
Injected n=2; suppository n=3				

EXAMPLE 18

[0147] Experiments were carried out using rats with the w/o microemulsion of this invention to evaluate them as a vehicle for the delivery of the peptide cyclo(S,S)-N^{α}-acetyl-Cys-(N^{α}-methyl) Arg-Gly-Asp-Pen-NH_2.

Formulations

[0148] The test microemulsion systems were prepared according to the methods of the application with the peptide added to the system last.

COMPOSITIONS OF THE MICROEMULSIONS (WEIGHT %)						
COMPONENT (WT%)	ME-1	ME-2	ME-3	ME-4	ME-5	ME-6
CAPTEX® 200	68.30	76.47		76.57	76.65	76.49
MYVACET®			76.91			
CAPMUL® MCM	8.31		9.09		9.28	9.26
DICAPRIN®				9.26		
CENTROPHASE® 31	1.60	1.61		0.96	2.13	
MYVEROL® 18-92			1.04			1.06
CREMOPHOR® EL	16.52	16.63	9.82	10.01		10.00
TWEEN® 80					8.74	
SALINE (PEPTIDE)	5.26	5.30	3.13	3.19	3.20	3.19

Test Method

[0149] Intravenous (i.v.) Administration: Fasted rats were anesthetized with an intraperitoneal (i.p.) injection and surgically fitted with a jugular catheter (ACUC protocol #90-151). Rats were allowed to recover from the surgery for 1 day. Catherized rats were fasted for 18 hr prior to the experiment. Each rot received either a 1 mg or 3 mg peptide/kg dose by lateral tail-vein administration. Blood samples of 0.5 ml aliquots were collected at 0, 1, 3, 5, 10, 15, 30, 45, 60, 90, 120, 150, and 180 min. The 0 min sample was taken 15 min prior to administration of the dose. Plasma was removed from the whole blood by centrifugation at 1600 x g for 5 min, and then plasma was stored at -20°C in 250 µl aliquots per sample. The blood pellet was reconstituted with 12.5 units heparinized saline and returned to the appropriate rat via the jugular catheter. After the experiment, rats were euthanized with i.v. administration of pentobarbital.

[0150] Intraduodenal (i.d.) Adminstration: Fasted rats were administered an i.p. injection of anesthesia cocktail and surgically fitted with jugular and duodenal catheters. Rats were allowed to recover from the surgery for 4-5 days (ACUC protocol #91-055). Catherized rats were fasted 18-20 hr prior to the experiment. Each group of rats received either 10 mg peptide/kg in each microemulsion (3.3 ml/kg) or 6.5 mg peptide/kg in each microemulsion (3.3 ml/kg). A saline control was administered to a group of rats containing 10 mg peptide/kg in a saline solution. Blood samples of 0.5 ml aliquots were collected via jugular catheter in heparinized eppendorf tubes at 0, 10, 30, 60, 120, 180, 240, and 1440 min. The 0 min sample was taken 15 min prior to administration of the dose by duodenal catheter. Plasma was collected for analysis and the blood returned to rats as described in the i.v. administration protocol. After 24 hr, rats were euthanized by i.v. administration of pentobarbital, exsanguinated, and a macroscopic observation of the intestinal tract was performed.

[0151] Post-Column HPLC Fluorescence Assay: For samples and standards, plasma components were precipitated with 0.6 ml cetonitrile, and then pelleted by centrifugation at 16,000 x g for 20 min. The supermatant was removed, and then dried to powder under N_2 at 40°C. Powder was dissolved in 0.5 ml 1% TFA solution, and then processed by solid-phase extraction procedure (SPEP). SPEP was as follows: 1) condition 1 ml C_{18} columns with methanol, and then rinse columns with 1 ml water, 2) standards and samples were applied to columns, and then rinsed twice with 1 ml water, 3) standards and samples were collected in tubes upon elution from column with methanol by two 0.5 ml aliquots. The samples and standards were dried to powder under N_2 at 40°C, and then dissolved in 100 µl of 10% methanol: 90% ultrapure water solution. Standards and samples were placed in HPLC vials. Vials with standards were placed before and after vials containing the samples for HPLC analysis. For the peptide standards, an aliquot was injected for analysis

based on the concentration of the standard as follows: 50 µl aliquot was injected for analysis by post-column fluorescence detection. Fluorescence chromatography data were collected and integrated using Nelson Chromatography Data System. The peak area ratio (Y) and peptide standard concentration (X) were used to determine the slope of a line which was forced through the origin from the equation: slope=(sum of X*Y)/(sum of X^2) . The slope represented the relationship between peak area ratio and peptide plasma concentration for the samples.

Results

[0152] The area under the plasma concentration curve (AUC) was determined for each test group. The percentage bioavailability was determined by the equation with the average AUC from iv administration: $[(AUC_{id}/AUC_{iv})*(mg/kg_{iv}/mg/kg_{id})]*100$. The summary of the results are listed below in which the microemulsion formulations of the present invention showed a significant increase in the bioavailability of the peptide in comparison to the saline solution.

FORMULATION	DOSE (mg/kg)	N	AUC[1]	BAC[2](%)
SALINE	10.0	3	0.011 ± .005	0.5 ± 0.3
ME-1	6.5	3	0.405 ± .099	29.1 ± 7.1
ME-2	6.5	3	0.269 ± 0.164	19.4 ± 11.8
ME-3	10.0	3	0.115 ± 0.042	5.4 ± 2.2
ME-4	10.0	3	0.054 ± 0.04	2.5 ± 1.9
ME-5	10.0	1	0.8	7.4
ME-6	10.0	3	0.308 ± 0.094	14.4 ± 4.4

[1] Area under the curve (mg*min/ml)
[2] Bioavailability relative to i.v. injected peptide

EXAMPLE 19

[0153] A w/o microemulsion according to ME-1 from Example 18 was formulated with the growth hormone releasing peptide His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2. The composition of the microemulsion was:

Captex 200	68.3% w/w
Capmul MCM	8.3% w/w
Centrophase 31	1.6% w/w
Cremophor EL	16.5% w/w
Aqueous	5.3% w/w

[0154] The aqueous solution contained 25.43 mg peptide/ml.

EXAMPLE 20-24

[0155] A w/o microemulsion according to ME-2, ME-3, ME-4, ME-5, and ME-6 from Example 18 is formulated with the growth hormone releasing peptide His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 at both about 25mg/ml and 75 mg/ml of the aqueous medium.

EXAMPLE 25

[0156] Various phase diagrams were prepared by mixing the surfactants in the weight ratios indicated in the following figures and then mixing the surfactant mixture with the oil in various weight ratios. The oil/surfactant mixtures were then titrated with increasing amounts of a 0.9% w/w saline solution. The experiments were carried out at room temperature, 22-23°C, unless indicated otherwise. The water-in-oil microemulsion regions were stable for at least 24 hours as determined by maintaining a single phase system. The presence of liquid crystalline phases was determined by examination of the samples between crossed polarizers, these systems were not defined in the figures as water-in-oil microemul-

sions.

[0157] The components of the water-in-oil microemulsions are:

Captex® 200 -	propylene glycol esters of capric/caprylic acids (Karlshamas Lipid Specialties, Columbus, OH)
Capmul® MCM -	mono-2nd diglycerides of medium-chain fatty acids (capric and caprylic) (Karlshamas Lipid Specialties, Columbus, OH) (HLB=5.0)
Cremophor® EL -	polyoxyethylene glycerol triricinoleate 35 DAC (BASF, Inc.) (HLB=13.5)
Myverol® 18-92 -	glycerol monolinoleate (HLB=3.8-4.0)
Centrophase® 31 -	lecithin (mol. wt. - 800) (Central Soya, Fort Wayne, IN) (HLB=4.0)
Tween® 80 -	polyoxyethylene-sorbitan monooleate, Sigma Corp. (HLB=15)
Whitepsol® H-15 -	a 90:10% Wt. mixture of triesters: diesters of glycerol and lauric acid with less than 2% wt. monoglycerides, m.p. 33-36°C.

[0158] In FIG. 1, the region defined as "A" is the water-in-oil microemulsion region while the region defined as "B" is a micelle solution region. In Fig. 1, the oil is Captex® 200, the aqueous phase is a 0.9% wt. NaCl aqueous solution, and the surfactant mixture is Capmul MCM:Myverol® 18-92:Cremophor® EL in a weight ratio of 45.5:5.2:49.2. ME-6 from Example 18 is included within this phase diagram.

[0159] In Fig. 2 the oil is Captex® 200, the aqueous phase is 0.9% wt. NaCl aqueous solution, and the surfactant mixture is Capmul® MCM:Centrophase® 31:Tween® 80 in a weight ratio of 46:10.6:43.4.

[0160] In Fig. 3 the oil is Captex® 200, the aqueous phase is 0.9% wt. NaCl aqueous solution, and the surfactant mixture is Capmul® MCM:Centrophase® 31:Cremophor® EL in a weight ratio of 31.5:6:62.5. This system includes the ME-1 used in Example 18.

[0161] In Fig. 4 the oil is Whitepsol® H-15, the aqueous phase is a 20% wt. Sorbitol in 0.9% wt. NaCl aqueous solution, and the surfactant mixture is Capmul® MCM:Myverol 18-92:Tween® 80 in a weight ratio of 15.4:8.5:76.

[0162] In Fig. 5 the oil is MYVACET® 9-45K, the aqueous phase is 0.9% wt. NaCl aqueous solution, and the surfactant mixture is Capmul® MCM:Myverol® 18-92:Cremophor® EL in a weight ratio of 45.5;5.2:49.2.

EXAMPLE 26

[0163] Water-in-oil microemulsions depicted in Figs. 1-5 can be made using both about 25 mg peptide/ml and 75 mg peptide/ml aqueous phase using both peptides cyclo(S,S)-N^{α}-acetyl-Cys-(N^{α}-methyl)Arg-Gly-Asp-Pen-NH_2) and His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2).

Claims

1. A liquid water-in-oil microemulsion composition that converts to an oil-in-water emulsion by the addition of water, comprising:

   (a) up to 60 volume percent, based upon the total volume of the microemulsion, of an internally dispersed aqueous phase comprising an effective amount of a biologically-active, therapeutic water-soluble material;
   (b) from 5 to 99 volume percent of a continuous oil phase comprising propylene glycol diesters having from 7 to 55 carbon atoms; and
   (c) from 1 to 70 volume percent of a surfactant or mixture of surfactants, wherein the surfactant or surfactant mixture has a hydrophilic-lipophilic balance value of from 7 to 14,
   wherein the biologically-active material is selected from the group consisting of calcitonins, insulins, fibrinogen antagonists, growth hormone releasing peptides, interleukins, erythropoietins, colony stimulating factors, RGD peptides, hematoregulatory peptides, vasopressin, collagenase inhibitors, angiotensin inhibitors, mammalian growth hormones, heparins, clotting factors, hypothalamic releasing peptides, and tumor necrosis factor, and wherein the water:oil partition coefficient of the biologically-active material is greater than 10:1.

2. The composition of claim 1 wherein the oil phase comprises diesters of propylene glycol having from 15 to 40 carbon atoms.

3. A liquid water-in-oil microemulsion composition that converts to an oil-in-water emulsion by the addition of water, comprising:

   (a) up to 60 volume percent, based upon the total volume of the microemulsion, of an internally dispersed aqueous phase comprising an effective amount of a biologically-active, therapeutic, water-soluble material;

(b) from 5 to 99 volume percent of a continuous oil phase comprising diesters of propylene glycol having from 15 to 40 carbon atoms; and

(c) from 1 to 70 volume percent of a surfactant or mixture of surfactants, wherein the surfactant or surfactant mixture has a hydrophilic-lipophilic balance value of from 7 to 14,

wherein the active material has a water:oil partition coefficient greater than 10:1.

4. The composition of claim 3 wherein the active material is a protein, peptide, immunogen, or other pharmaceutically-active material.

5. A water-in-oil microemulsion composition that is a solid at temperatures of 22-23°C and that converts to an oil-in-water emulsion by the addition of water comprising:

(a) up to 60 volume percent, based upon the total volume of the microemulsion, of an internally dispersed aqueous phase comprising an effective amount of a biologically-active, water-soluble material;

(b) from 5 to 99 volume percent of a continuous oil phase comprising diesters of propylene glycol having from 7 to 55 carbon atoms; and

(c) from 1 to 70 volume percent of a surfactant or mixture of surfactants, wherein the surfactant or surfactant mixture has a hydrophilic-lipophilic balance value of from 7 to 14,

wherein the active material has a water:oil partition coefficient greater than 10:1, and wherein the oil phase, surfactant or mixture of surfactants, or both, comprises a component that has a melting point above about 23°C.

6. The composition of claim 5 wherein the active material is a therapeutic and is a protein, peptide, immunogen, or other pharmaceutically-active material.

7. The composition of claim 6 wherein the Oil phase comprises a triglyceride having at least 45 carbon atoms, and propylene glycol diester having at least 31 carbon atoms, or mixtures thereof.

8. The composition of claim 6 wherein the continuous oil phase is formulated with an oil that has a melting point above 22-23°C.

9. A water-in-oil microemulsion composition that converts to an oil-in-water microemulsion upon the addition of water, comprising:

(a) up to 60 volume percent, based upon the total of the microemulsion, of an internally dispersed aqueous phase comprising an effective amount of a biologically-active, water-soluble material consisting of therapeutic water-soluble materials having a water: oil partition coefficient greater than 10:1, and a modifier comprising sorbitol, polyethylene glycol, propylene glycol, mannitol, monosaccharides, or disaccharides, prsent in an amount of from 10-50% by weight of the aqueous phase and sufficient to cause the water-in-oil microemulsion to convert to an oil-in-water microemulsion upon the addition of aqueous medium;

(b) from 5 to 99 volume percent of a continuous oil phase comprising at lest one pharmaceutically acceptable oil; and

(c) from 1 to 70 volume percent of a surfactant or mixture of surfactants, wherein the surfactant or mixture of surfactants has a hydrophilic-lipophilic balance value of greater than 7.

10. The Composition of claim 9 wherein the active material is a protein, peptide, immunogen, or other pharmaceutically-active material.

11. The composition of any of claims 1-10 wherein the aqueous phase is up to 20 percent by weight of the water-in-oil microemulsion.

12. A composition according to any of the claims 1 to 11 wherein the surfactant or mixture of surfactants comprises cetyldimethylethylammonium bromide, cetylpyridinium chloride; C_{8-32} fatty acids and salts thereof; cholic acid and derivatives thereof; C_{8-56} diesters of tartaric acid; phospholipids; C_{5-29} monoesters of lactic acid; C_{8-20} sulfonates, including alkyl-, olefin-, and alkylaryl derivatives; tridecyl- and dodecylbenzene sulfonic acids; C_{5-33} sarcosine and betaine derivatives; phosphatidylethanolamine, sphingomyelins, ethoxylated castor oil; C_{5-29} monoglycerides and ethoxylated derivatives thereof; C_{15-60} diglycerides and polyoxyethylene derivatives thereof having 1 to 90 POE groups; C_{10-40} esters of long chain fatty acids; C_{10-40} alcohols; C_{8-96} ethoxylated fatty esters; C_{14-130} sucrose fatty

esters; and C_{20-130} sorbitol and sorbitan monoesters, diesters, and triesters, or polyoxyethylene (POE) derivatives thereof having 1 to 90 POE groups.

13. A composition according to any of the claims 1 - 11 wherein the surfactant or mixture of surfactants comprises C_{9-13} monoglycerides.

14. A composition according to any of the claims 1-11 wherein the oil phase comprises diesters of propylene glycol having from 19 to 23 carbon atoms and the surfactant or mixtures of surfactants comprises C_{9-13} monoglycerides.

15. A composition according to any of the claims 1-11 wherein the hydrophilic-lipophllic balance of the surfactant or surfactant mixture is from 8 to 13.

16. The composition of any of the claims 1-11 wherein the surfactant or mixture of surfactants comprises C_{9-13} monoglycerides, C_{15-60} diglycerides, C_{8-96} ethoxylated fatty esters, C_{20-130} sorbitol and sorbitan monoesters, diesters, and triesters, or polyoxyethylene (POE) derivatives thereof having 1 to 90 POE groups.

17. A composition according to any preceding claim wherein the aqueous phase is from 30 to 60 volume percent of the water-in-oil microemulsion.

18. A composition according to any preceding claim wherein the active material is a protein or a peptide.

19. A composition according to any preceding claim wherein the active material is a fibrinogen antagonist.

20. A composition according to claim 19 wherein the active material is a peptide having the sequence cyclo (S,S)-N^{α}-acetyl-Cys-(N^{α}-methyl)Arg-Gly-Asp-Pen-NH_2.

21. A composition according to any of the claims 1-12 wherein the active material is a growth hormone releasing peptide.

22. A composition according to claim 21 wherein the active materials is a peptide having the sequence His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2.

23. A composition according to any of the claims 1-11 wherein the active material is selected from the group consisting of calcitonins, insulins, and human growth hormones.

24. A biologically compatible water-in-oil microemulsion composition which converts to an oil-in-water emulsion by the addition of water, comprising:

(a) up to 60 volume percent of an internal dispersed aqueous phase comprising an effective amount of a biologically active water soluble material;
(b) from 5 to 99 volume percent of a continuous oil phase comprising at least one pharmaceutically-acceptable oil; and
(c) from 1 to 70 volume percent of a surfactant or surfactant mixture comprising one or more C_{8-32} fatty acids or salts thereof, wherein the surfactant or surfactant mixture has an HLB value of least 7.

25. The water-in-oil microemulsion composition of claim 24 wherein the biologically active material is a therapeutic and is a protein, peptide, immunogen, or other pharmaceutically active material and the water: oil partition coefficient of the active material is greater than 10:1.

26. The water-in-oil microemulsion composition of claim 25 wherein the biologically active material is a protein or a peptide.

27. The water-in-oil microemulsion composition of claim 24 wherein the oil phase comprises a triester of glycerol having 20-60 carbon atoms, a C_{15-40} diester of propylene glycol, or mixtures thereof.

28. The water-in-oil microemulsion composition of claim 24 comprising: from 0.1 to 15 percent by volume of the aqueous phase; from 50 to 90% by volume of the continuous oil phase, and from 2 to 50% by volume of the surfactant.

29. The composition of any preceding claim for use in therapy.

30. Use of a water-in-oil microemulsion composition that converts to an oil-in-water emulsion by the addition of water in the preparation of a medicament for use in therapy comprising the administration of an effective amount of the medicament to the body of an animal wherein the administration is either parentally, enterally or via any mucous membrane, wherein the microemulsion composition comprises:

(a) up to 60 volume percent, based upon the total volume of the microemulsion of an internally dispersed aqueous phase comprising an effective amount of a biologically-active material having a water:oil partition coefficient of greater than 10:1;
(b) from 5 to 99 volume percent of a continuous oil phase comprising diesters of propylene glycol having from 7 to 55 carbon atoms, and
(c) from about 1 to 70 volume percent of a surfactant or mixture of surfactants wherein the surfactant or surfactant mixture has a hydrophilic-lipophilic balance value of from 7 to 14.

31. Use of a water-in-oil microemulsion composition that converts to an oil-in-water emulsion by the addition of water in the preparation of a medicament for use in therapy comprising the administration of an effective amount of the medicament to the body of an animal wherein the administration is oral, wherein the microemulsion composition comprises:

(a) up to 60 volume percent, based upon the total volume of the microemulsion, of an internally dispersed aqueous phase comprising an effective amount of a biologically-active material having a water:oil partition coefficient of greater than 10:1;
(b) from 5 to 99 volume percent of a continuous oil phase comprising at least one pharmaceutically-acceptable oil; and
(c) from 1 to 70 volume percent of a surfactant or mixture of surfactants comprising C_{9-13} monoglycerides, C_{15-23} diglycerides, and mixtures thereof, and wherein the surfactant or surfactant mixture has a hydrophilic-lipophilic balance value of from 7 to 14.

32. Use of a water-in-oil microemulsion composition that converts to an oil-in-water emulsion by the addition of water in the preparation of a medicament for use in therapy comprising the administration of an effective amount of the medicament to the body of an animal wherein the administration is rectal, wherein the microemulsion composition comprises:

(a) up to 60 volume percent, based upon the total volume of the microemulsion, of an internally dispersed aqueous phase comprising an effective amount of a biologically-active material having a water:oil partition coefficient of greater than 10:1;
(b) from 5 to 99 volume percent of a continuous oil phase; and
(c) from 1 to 70 volume percent of a surfactant or mixture of surfactants, wherein the surfactant or surfactant mixture has a hydrophilic-lipophilic balance value of from 7 to 14.

33. Use of a water-in-oil microemulsion composition in the preparation of a medicament for use in therapy comprising the administration of an effective amount of the medicament to the body of an animal wherein the administration is either parenteral, enteral or via any other mucous membrane, and further comprising converting the water-in-oil microemulsion to an oil-in-water microemulsion after the administration step by the addition of aqueous body fluid, wherein the microemulsion composition comprises:

(a) up to 60 volume percent, based upon the total volume of the microemulsion, of an internally dispersed aqueous phase comprising an effective amount of a biologically-active material having a water:oil partition coefficient of greater than 10:1, and a modifier, present in an amount sufficient to cause the water-in-oil microemulsion to convert to an oil-in-water microemulsion upon the addition of aqueous medium;
(b) from 5 to 99 volume percent of a continuous oil phase comprising at least one pharmaceutically-acceptable oil; and
(c) from 1 to 70 volume percent of a surfactant or mixture of surfactants, wherein the surfactant or surfactant mixture has a hydrophilic-lipophilic balance value of at least 7.

34. Use according to claim 32 wherein the composition is a solid.

35. Use according to claim 30 or 33 wherein the administration is oral.

36. Use according to any of the claims 30, 31, 32 or 34 further comprising converting the water-in-oil microemulsion to an oil-in-water microemulsion.

37. Use according to any one of claims 30, 31, 33, 35 or 36 wherein the water-in-oil microemulsion is a liquid.

38. Use according to any of the claims 30 to 37, wherein the biologically-active material is a therapeutic protein or a peptide.

39. Use according to any of the claims 30 to 38 wherein the biologically-active material is either a fibrinogen antagonist, a growth hormone releasing peptide, calcitonin, insulin, or a human growth hormone.

Patentansprüche

1. Eine flüssige Wasser-in-Öl-Mikroemulsionszusammensetzung, die sich durch den Zusatz von Wasser in eine Öl-in-Wasser-Emulsion umwandelt, enthaltend:

   (a) bis zu 60 Volumsprozent, bezogen auf das Gesamtvolumen der Mikroemulsion, eine intern dispergierte wässerige Phase, die eine wirksame Menge eines biologisch aktiven therapeutischen wasserlöslichen Materials enthält;
   (b) 5 bis 99 Volumsprozent kontinuierliche Ölphase, die Propylenglykol-Diester mit 7 bis 55 Kohlenstoffatomen enhält; und
   (c) 1 bis 70 Volumsprozent Tensid oder eine Mischung von Tensiden, wobei das Tensid oder die Mischung der Tenside einen hydrophilen-lipophilen Gleichgewichtswert von 7 bis 14 hat,
   wobei das biologisch aktive Material ausgewählt ist aus der Gruppe, die aus Calcitoninen, Insulinen, Fibrinogen-Antagonisten, Wachstumshormone freisetzenden Peptiden, Interleukinen, Erythropoietinen, koloniestimulierenden Faktoren, RGD-Peptiden, hämatoregulatorischen Peptiden, Vasopressin, Collagenase-Inhibitoren, Angiotensin-Inhibitoren, Säugetier-Wachstumshormonen, Heparinen, Gerinnungsfaktoren, hypothalamischen Freisetzungspeptiden und Tumornekrose-Faktor besteht, und wobei der Wasser:Öl-Verteilungskoeffizient des biologisch aktiven Materials größer als 10:1 ist.

2. Die Zusammensetzung nach Anspruch 1, in welcher die Ölphase Diester von Propylenglykol mit 15 bis 40 Kohlenstoffatomen enthält.

3. Eine flüssige Wasser-in-Öl-Mikroemulsionszusammensetzung, die sich durch den Zusatz von Wasser in eine Öl-in-Wasser-Emulsion umwandelt, enthaltend:

   (a) bis zu 60 Volumsprozent, bezogen auf das Gesamtvolumen der Mikroemulsion, eine intern dispergierte wässerige Phase, die eine wirksame Menge eines biologisch aktiven therapeutischen wasserlöslichen Materials enthält;
   (b) 5 bis 99 Volumsprozent kontinuierliche Ölphase, die Propylenglykol-Diester mit 15 bis 40 Kohlenstoffatomen enhält; und
   (c) 1 bis 70 Volumsprozent Tensid oder eine Mischung von Tensiden, wobei das Tensid oder die Tensidmischung einen hydrophilen-lipophilen Gleichgewichtswert von 7 bis 14 hat,
   wobei das aktive Material einen Wasser:Öl-Verteilungskoeffizienten von größer als 10:1 hat.

4. Die Zusammensetzung nach Anspruch 3, in welcher das aktive Material ein Protein, Peptid, Immunogen oder ein anderes pharmazeutisch aktives Material ist.

5. Eine Wasser-in-Öl-Mikroemulsionszusammensetzung, die bei Temperaturen von 22-23°C ein Feststoff ist und die sich durch den Zusatz von Wasser in eine Öl-in-Wasser-Emulsion umwandelt, enthaltend:

   (a) bis zu 60 Volumsprozent, bezogen auf das Gesamtvolumen der Mikroemulsion, eine intern dispergierte wässerige Phase, die eine wirksame Menge eines biologisch aktiven wasserlöslichen Materials enthält;
   (b) 5 bis 99 Volumsprozent kontinuierliche Ölphase, die Propylenglykol-Diester mit 7 bis 55 Kohlenstoffatomen enhält; und
   (c) 1 bis 70 Volumsprozent Tensid oder eine Mischung von Tensiden, wobei das Tensid oder die Tensidmi-

schung einen hydrophilen-lipophilen Gleichgewichtswert von 7 bis 14 hat,
wobei das aktive Material einen Wasser:Öl-Verteilungskoeffizienten von größer als 10:1 hat und wobei die Ölphase, das Tensid oder die Mischung der Tenside oder beide einen Bestandteil umfassen, der einen Schmelzpunkt oberhalb von etwa 23°C hat.

6. Die Zusammensetzung nach Anspruch 5, in welcher das aktive Material ein Therapeutikum ist und aus einem Protein, Peptid, Immunogen oder einem anderen pharmazeutisch aktiven Material besteht.

7. Die Zusammensetzung nach Anspruch 6, in welcher die Ölphase ein Triglycerid mit mindestens 45 Kohlenstoffatomen und Propylenglykol-Diester mit mindestens 31 Kohlenstoffatomen oder Mischungen hievon enthält.

8. Die Zusammensetzung nach Anspruch 6, in welcher die kontinuierliche Ölphase mit einem Öl formuliert ist, das einen Schmelzpunkt oberhalb von 22-23°C hat.

9. Eine Wasser-in-Öl-Mikroemulsionszusammensetzung, die sich durch den Zusatz von Wasser in eine Öl-in-Wasser-Mikroemulsion umwandelt, enthaltend:

(a) bis zu 60 Volumsprozent, bezogen auf das Gesamtvolumen der Mikroemulsion, eine intern dispergierte wässerige Phase, die eine wirksame Menge eines biologisch aktiven wasserlöslichen Materials, das aus therapeutischen wasserlöslichen Materialien mit einem Wasser:Öl-Verteilungskoeffizienten von größer als 10:1 besteht, und einen Modifikator enthält, der Sorbit, Polyethylenglykol, Propylenglykol, Mannit, Monosaccharide oder Disaccharide umfaßt und in einer Menge von 10-50 Gewichtsprozent der wässerigen Phase und in ausreichender Menge, um die Wasser-in-Öl-Mikroemulsion durch den Zusatz von wässerigem Medium in eine Öl-in-Wasser-Mikroemulsion umzuwandeln, vorliegt;
(b) 5 bis 99 Volumsprozent kontinuierliche Ölphase, die mindestens ein pharmazeutisch verwendbares Öl umfaßt; und
(c) 1 bis 70 Volumsprozent Tensid oder eine Mischung von Tensiden, wobei das Tensid oder die Mischung der Tenside einen hydrophilen-lipophilen Gleichgewichtswert von größer als 7 hat.

10. Die Zusammensetzung nach Anspruch 9, in welcher das aktive Material ein Protein, Peptid, Immunogen oder ein anderes pharmazeutisch aktives Material ist.

11. Die Zusammensetzung nach einem der Ansprüche 1 bis 10, in welcher die wässerige Phase bis zu 20 Gewichtsprozent der Wasser-in-Öl-Mikroemulsion ausmacht.

12. Eine Zusammensetzung nach einem der Ansprüche 1 bis 11, in welcher das Tensid oder die Mischung der Tenside Cetyldimethylethylammoniumbromid; Cetylpyridiniumchlorid; C_{8-32}-Fettsäuren und Salze hievon; Cholsäure und Derivate derselben; C_{8-56}-Diester der Weinsäure; Phospholipide; C_{5-29}-Monoester der Milchsäure; C_{8-20}-Sulfonate, inklusive Alkyl-, Olefin- und Alkylaryl-Derivate; Tridecyl- und Dodecylbenzolsulfonsäuren; C_{5-33}-Sarcosin- und -Betain-Derivate; Phosphatidylethanolamin, Sphingomyeline, ethoxyliertes Rizinusöl; C_{5-29}-Monoglyceride und ethoxylierte Derivate hievon; C_{15-60}-Diglyceride und Polyoxyethylen-Derivate hievon mit 1 bis 90 POE-Gruppen; C_{10-40}-Ester langkettiger Fettsäuren; C_{10-40}-Alkohole; C_{8-96}-ethoxylierte Fettsäureester; C_{14-130}-Saccharose-Fettsäureester und C_{20-130}-Sorbit- und -Sorbitanmonoester, -diester und -triester oder Polyoxyethylen- (POE)-Derivate hievon mit 1 bis 90 POE-Gruppen enthält.

13. Eine Zusammensetzung nach einem der Ansprüche 1 bis 11, in welcher das Tensid oder die Mischung der Tenside C_{9-13}-Monoglyceride enthält.

14. Eine Zusammensetzung nach einem der Ansprüche 1 bis 11, in welcher die Ölphase Diester von Propylenglykol mit 19 bis 23 Kohlenstoffatomen enthält und das Tensid oder die Mischungen der Tenside C_{9-13}-Monoglyceride enthält.

15. Eine Zusammensetzung nach einem der Ansprüche 1 bis 11, in welcher das hydrophile-lipophile Gleichgewicht des Tensids oder der Tensidmischung 8 bis 13 beträgt.

16. Die Zusammensetzung nach einem der Ansprüche 1 bis 11, in welcher das Tensid oder die Mischung der Tenside C_{9-13}-Monoglyceride, C_{15-60}-Diglyceride, C_{8-96}-ethoxylierte Fettsäureester, C_{20-130}-Sorbit- oder -Sorbitanmonoester, -diester und -triester oder Polyoxyethylen-(POE)- Derivate hievon mit 1 bis 90 POE-Gruppen enthält.

17. Eine Zusammensetzung nach einem der vorhergehenden Ansprüche, in welcher die wässerige Phase 30 bis 60 Volumsprozent der Wasser-in-Öl-Mikroemulsion ausmacht.

18. Eine Zusammensetzung nach einem der vorhergehenden Ansprüche, in welcher das aktive Material ein Protein oder ein Peptid ist.

19. Eine Zusammensetzung nach einem der vorhergehenden Ansprüche, in welcher das aktive Material ein Fibrinogen-Antagonist ist.

20. Eine Zusammensetzung nach Anspruch 19, in welcher das aktive Material ein Peptid ist, das die Sequenz Cyclo-(S,S)-N^{α}-acetyl-Cys-(N^{α}-methyl)Arg-Gly-Asp-Pen-NH_2 hat.

21. Eine Zusammensetzung nach einem der Ansprüche 1 bis 12, in welcher das aktive Material ein ein Wachstumshormon freisetzendes Peptid ist.

22. Eine Zusammensetzung nach Anspruch 21, in welcher das aktive Material ein Peptid mit der Sequenz His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 ist.

23. Eine Zusammensetzung nach einem der Ansprüche 1 bis 11, in welcher das aktive Material aus der aus Calcitoninen, Insulinen und humanen Wachstumshormonen bestehenden Gruppe ausgewählt ist.

24. Eine biologisch kompatible Wasser-in-Öl-Mikroemulsionszusammensetzung, die sich durch den Zusatz von Wasser in eine Öl-in-Wasser-Emulsion umwandelt, enthaltend:

    (a) bis zu 60 Volumsprozent eine interne dispergierte wässerige Phase, die eine wirksame Menge eines biologisch aktiven wasserlöslichen Materials enthält;
    (b) 5 bis 99 Volumsprozent kontinuierliche Ölphase, die mindestens ein pharmazeutisch verwendbares Öl enthält; und
    (c) 1 bis 70 Volumsprozent Tensid oder eine Tensidmischung, die eine oder mehrere C_{8-32}-Fettsäuren oder Salze hievon enthält, wobei das Tensid oder die Tensidmischung einen HLB-Wert von mindestens 7 hat.

25. Die Wasser-in-Öl-Mikroemulsionszusammensetzung nach Anspruch 24, in welcher das biologisch aktive Material ein Therapeutikum ist und aus einem Protein, Peptid, Immunogen oder einem anderen pharmazeutisch aktiven Material besteht und der Wasser:Öl-Verteilungskoeffizient des aktiven Materials größer als 10:1 ist.

26. Die Wasser-in-Öl-Mikroemulsionszusammensetzung nach Anspruch 25, in welcher das biologisch aktive Material ein Protein oder ein Peptid ist.

27. Die Wasser-in-Öl-Mikroemulsionszusammensetzung nach Anspruch 24, in welcher die Ölphase einen Triester von Glycerin mit 20-60 Kohlenstoffatomen, einen C_{15-40}-Diester von Propylenglykol oder Mischungen hievon enthält.

28. Die Wasser-in-Öl-Mikroemulsionszusammensetzung nach Anspruch 24, die enthält: 0,1 bis 15 Volumsprozent wässerige Phase; 50 bis 90 Volumsprozent kontinuierliche Ölphase und 2 bis 50 Volumsprozent Tensid.

29. Die Zusammensetzung nach einem der vorhergehenden Ansprüche zur Verwendung in der Therapie.

30. Verwendung einer Wasser-in-Öl-Mikroemulsionszusammensetzung, die sich durch den Zusatz von Wasser in eine Öl-in-Wasser-Emulsion umwandelt, bei der Herstellung eines Medikaments zur therapeutischen Verwendung, bei welcher die Verabreichung einer wirksamen Menge des Medikaments an den Körper eines Tieres stattfindet und die Verabreichung parenteral, enteral oder über eine Schleimhaut erfolgt, wobei die Mikroemulsionszusammensetzung enthält:

    (a) bis zu 60 Volumsprozent, bezogen auf das Gesamtvolumen der Mikroemulsion, eine intern dispergierte wässerige Phase, die eine wirksame Menge eines biologisch aktiven Materials mit einem Wasser:Öl-Verteilungskoeffizienten von größer als 10:1 enthält;
    (b) 5 bis 99 Volumsprozent kontinuierliche Ölphase, die Propylenglykol-Diester mit 7 bis 55 Kohlenstoffatomen enhält; und
    (c) etwa 1 bis 70 Volumsprozent Tensid oder eine Mischung von Tensiden, wobei das Tensid oder die Tensid-

mischung einen hydrophilen-lipophilen Gleichgewichtswert von 7 bis 14 hat.

31. Verwendung einer Wasser-in-Öl-Mikroemulsionszusammensetzung, die sich durch den Zusatz von Wasser in eine Öl-in-Wasser-Emulsion umwandelt, bei der Herstellung eines Medikaments zur Verwendung in der Therapie, bei welcher die Verabreichung einer wirksamen Menge des Medikaments an den Körper eines Tieres stattfindet und die Verabreichung oral erfolgt, wobei die Mikroemulsionszusammensetzung enthält:

(a) bis zu 60 Volumsprozent, bezogen auf das Gesamtvolumen der Mikroemulsion, eine intern dispergierte wässerige Phase, die eine wirksame Menge eines biologisch aktiven Materials mit einem Wasser:Öl-Verteilungskoeffizienten von größer als 10:1 enthält;
(b) 5 bis 99 Volumsprozent kontinuierliche Ölphase, die mindestens ein pharmazeutisch verwendbares Öl enthält; und
(c) 1 bis 70 Volumsprozent Tensid oder eine Mischung von Tensiden, das bzw. die C_{9-13}-Monoglyceride, C_{15-23}-Diglyceride und Mischungen hievon enthält, wobei das Tensid oder die Tensidmischung einen hydrophilen-lipophilen Gleichgewichtswert von 7 bis 14 hat.

32. Verwendung einer Wasser-in-Öl-Mikroemulsionszusammensetzung, die sich durch den Zusatz von Wasser in eine Öl-in-Wasser-Emulsion umwandelt, bei der Herstellung eines Medikaments zum Einsatz in der Therapie, bei welcher die Verabreichung einer wirksamen Menge des Medikaments an den Körper eines Tieres stattfindet und die Verabreichung rectal erfolgt, wobei die Mikroemulsionszusammensetzung enthält:

(a) bis zu 60 Volumsprozent, bezogen auf das Gesamtvolumen der Mikroemulsion, eine intern dispergierte wässerige Phase, die eine wirksame Menge eines biologisch aktiven Materials mit einem Wasser:Öl-Verteilungskoeffizienten von größer als 10:1 enthält;
(b) 5 bis 99 Volumsprozent kontinuierliche Ölphase; und
(c) 1 bis 70 Volumsprozent Tensid oder eine Mischung von Tensiden, wobei das Tensid bzw. die Tensidmischung einen hydrophilen-lipophilen Gleichgewichtswert von 7 bis 14 hat.

33. Verwendung einer Wasser-in-Öl-Mikroemulsionszusammensetzung bei der Herstellung eines Medikaments zum Einsatz in der Therapie, bei welcher die Verabreichung einer wirksamen Menge des Medikaments an den Körper eines Tieres stattfindet und die Verabreichung parenteral, enteral oder über irgendeine andere Schleimhaut erfolgt und wobei sich weiters die Wasser-in-Öl-Mikroemulsion nach dem Verabreichungsschritt durch den Zusatz von wässerigem Körperfluid in eine Öl-in-Wasser-Mikroemulsion umwandelt, wobei die Mikroemulsionszusammensetzung enthält:

(a) bis zu 60 Volumsprozent, bezogen aufdas Gesamtvolumen der Mikroemulsion, eine intern dispergierte wässerige Phase, die eine wirksame Menge eines biologisch aktiven Materials mit einem Wasser:Öl-Verteilungskoeffizienten von größer als 10:1 und einen Modifikator enthält, der in ausreichender Menge vorliegt, um zu bewirken, daß die Wasser-in-Öl-Mikroemulsion durch den Zusatz von wässerigem Medium in eine Öl-in-Wasser-Mikroemulsion umgewandelt wird;
(b) 5 bis 99 Volumsprozent kontinuierliche Ölphase, die mindestens ein pharmazeutisch verwendbares Öl umfaßt; und
(c) 1 bis 70 Volumsprozent Tensid oder eine Mischung von Tensiden, wobei das Tensid oder die Tensidmischung einen hydrophilen-lipophilen Gleichgewichtswert von mindestens 7 hat.

34. Verwendung nach Anspruch 32, wobei die Zusammensetzung ein Feststoff ist.

35. Verwendung nach Anspruch 30 oder 33, wobei die Verabreichung oral efolgt.

36. Verwendung nach einem der Ansprüche 30, 31, 32 oder 34, wobei weiters die Wasser-in-Öl-Mikroemulsion in eine Öl-in-Wasser-Mikroemulsion umgewandelt wird.

37. Verwendung nach einem der Ansprüche 30, 31, 33, 35 oder 36, wobei die Wasser-in-Öl-Mikroemulsion eine Flüssigkeit ist.

38. Verwendung nach einem der Ansprüche 30 bis 37, wobei das biologisch aktive Material ein therapeutisches Protein oder ein Peptid ist.

39. Verwendung nach einem der Ansprüche 30 bis 38, wobei das biologisch aktive Material ein Fibrinogen-Antagonist, ein ein Wachstumshormon freisetzendes Peptid, Calcitonin, Insulin oder ein humanes Wachstumshormon ist.

Revendications

1. Composition liquide de microémulsion d'eau dans l'huile qui se transforme en une émulsion d'huile dans l'eau par addition d'eau, comprenant:

   (a) jusqu'à 60 pour cent en volume, basé sur le volume total de la microémulsion, d'une phase aqueuse à dispersion interne comprenant une quantité efficace d'une substance thérapeutique soluble dans l'eau, biologiquement active;
   (b) de 5 à 99 pour cent en volume d'une phase huileuse continue comprenant des diesters de propylèneglycol ayant de 7 à 55 atomes de carbone; et
   (c) de 1 à 70 pour cent en volume d'un tensioactif ou d'un mélange de tensioactifs, le tensioactif ou le mélange de tensioactifs ayant une balance hydrophile-lipophile de 7 à 14,
   dans laquelle la substance biologiquement active est choisie dans le groupe constitué par les calcitonines, les insulines, les antagonistes du fibrinogène, les peptides de libération des hormones de croissance, les interleukines, les érythropoïétines, les facteurs stimulant la formation de colonies, les peptides RGD, les peptides hématorégulateurs, la vasopressine, les inhibiteurs de collagénase, les inhibiteurs d'angiotensine, les hormones de croissance des mammifères, les héparines, les facteurs de coagulation, les peptides de libération hypothalamique et les facteurs nécrosant des tumeurs, et dans laquelle le coefficient de partage eau:huile de la substance biologiquement active est supérieur à 10:1.

2. Composition selon la revendication 1, dans laquelle la phase huileuse comprend des diesters de propylèneglycol ayant de 15 à 40 atomes de carbone.

3. Composition liquide de microémulsion d'eau dans l'huile qui se transforme en une émulsion d'huile dans l'eau par addition d'eau, comprenant:

   (a) jusqu'à 60 pour cent en volume, basé sur le volume total de la microémulsion, d'une phase aqueuse à dispersion interne comprenant une quantité efficace d'une substance thérapeutique soluble dans l'eau, biologiquement active;
   (b) de 5 à 99 pour cent en volume d'une phase huileuse continue comprenant des diesters de propylèneglycol ayant de 15 à 40 atomes de carbone; et
   (c) de 1 à 70 pour cent en volume d'un tensioactif ou d'un mélange de tensioactifs, le tensioactif ou le mélange de tensioactifs ayant une balance hydrophile-lipophile de 7 à 14,
   dans laquelle la substance active possède un coefficient de partage eau:huile supérieur à 10:1.

4. Composition selon la revendication 3, dans laquelle la substance active est une protéine, un peptide, un immunogène ou une autre substance active sur le plan pharmaceutique.

5. Composition de microémulsion d'eau dans l'huile qui est solide à des températures de 22-23°C et qui se transforme en une émulsion d'huile dans l'eau par addition d'eau, comprenant:

   (a) jusqu'à 60 pour cent en volume, basé sur le volume total de la microémulsion, d'une phase aqueuse à dispersion interne comprenant une quantité efficace d'une substance soluble dans l'eau, biologiquement active;
   (b) de 5 à 99 pour cent en volume d'une phase huileuse continue comprenant des diesters de propylèneglycol ayant de 7 à 55 atomes de carbone; et
   (c) de 1 à 70 pour cent en volume d'un tensioactif ou d'un mélange de tensioactifs, le tensioactif ou le mélange de tensioactifs ayant une balance hydrophile-lipophile de 7 à 14,
   dans laquelle la substance active possède un coefficient de partage eau:huile supérieur à 10:1, et dans laquelle la phase huileuse, le tensioactif ou le mélange de tensioactifs, ou les deux à la fois, comprennent un composant qui possède un point de fusion supérieur à environ 23°C.

6. Composition selon la revendication 5, dans laquelle la substance active est un agent thérapeutique et est une protéine, un peptide, un immunogène ou une autre substance active sur le plan pharmaceutique.

7. Composition selon la revendication 6, dans laquelle la phase huileuse comprend un triglycéride ayant au moins 45

atomes de carbone, et un diester de propylèneglycol ayant au moins 31 atomes de carbone, ou des mélanges de ceux-ci.

8. Composition selon la revendication 6, dans laquelle la phase huileuse continue est formulée avec une huile qui possède un point de fusion supérieur à 22-23°C.

9. Composition de microémulsion d'eau dans l'huile qui se transforme en une microémulsion d'huile dans l'eau après addition d'eau, comprenant:

(a) jusqu'à 60 pour cent en volume, basé sur le volume total de la microémulsion, d'une phase aqueuse à dispersion interne comprenant une quantité efficace d'une substance soluble dans l'eau, biologiquement active constituée de substances thérapeutiques solubles dans l'eau ayant un coefficient de partage eau:huile supérieur à 10:1, et un modificateur comprenant du sorbitol, du polyéthylèneglycol, du propylèneglycol, du mannitol, des monosaccharides ou des disaccharides, présents à raison de 10-50% en poids de la phase aqueuse et en quantité suffisante pour provoquer la transformation de la microémulsion d'eau dans l'huile en une microémulsion d'huile dans l'eau après addition d'un milieu aqueux;
(b) de 5 à 99 pour cent en volume d'une phase huileuse continue comprenant au moins une huile acceptable sur le plan pharmaceutique; et
(c) de 1 à 70 pour cent en volume d'un tensioactif ou d'un mélange de tensioactifs, le tensioactif ou le mélange de tensioactifs ayant une balance hydrophile-lipophile supérieure à 7.

10. Composition selon la revendication 9, dans laquelle la substance active est une protéine, un peptide, un immunogène ou une autre substance active sur le plan pharmaceutique.

11. Composition selon l'une quelconque des revendications 1 à 10, dans laquelle la phase aqueuse représente jusqu'à 20 pour cent en poids de la microémulsion d'eau dans l'huile.

12. Composition selon l'une quelconque des revendications 1 à 11, dans laquelle le tensioactif ou le mélange de tensioactifs comprend du bromure de cétyldiméthyléthylammonium, du chlorure de cétylpyridinium; des acides gras en C_{8-32} et leurs sels; de l'acide cholique et ses dérivés; des diesters en C_{8-56} d'acide tartrique; des phospholipides; des monoesters en C_{5-29} d'acide lactique; des sulfonates en C_{8-20}, notamment les dérivés alkyl-, oléfine- et alkylaryl-; des acides tridécyl- et dodécylbenzènesulfoniques ; des dérivés de sarcosine et de bétaïne en C_{5-33}; de la phosphatidyléthanolamine, des sphingomyélines, de l'huile de ricin éthoxylée; des monoglycérides en C_{5-29} et leurs dérivés éthoxylés; des diglycérides en C_{15-60} et leurs dérivés polyoxyéthylène ayant 1 à 90 groupes POE; des esters en C_{10-40} d'acides gras à chaîne longue; des alcools en C_{10-40}; des esters gras éthoxylés en C_{8-96}; des esters gras de saccharose en C_{14-130}; et des monoesters, diesters et triesters de sorbitane et de sorbitol en C_{20-130}, ou leurs dérivés polyoxyéthylène (POE) ayant 1 à 90 groupes POE.

13. Composition selon l'une quelconque des revendications 1 à 11, dans laquelle le tensioactif ou le mélange de tensioactifs comprend des monoglycérides en C_{9-13}.

14. Composition selon l'une quelconque des revendications 1 à 11, dans laquelle la phase huileuse comprend des diesters de propylèneglycol ayant de 19 à 23 atomes de carbone et le tensioactif ou le mélange de tensioactifs comprend des monoglycérides en C_{9-13}.

15. Composition selon l'une quelconque des revendications 1 à 11, dans laquelle la balance hydrophile-lipophile du tensioactif ou du mélange de tensioactifs est de 8 à 13.

16. Composition selon l'une quelconque des revendications 1 à 11, dans laquelle le tensioactif ou le mélange de tensioactifs comprend des monoglycérides en C_{9-13}, des diglycérides en C_{15-60}, des esters gras éthoxylés en C_{8-96}, des monoesters, diesters et triesters de sorbitane et de sorbitol en C_{20-130}, ou leurs dérivés polyoxyéthylène (POE) ayant 1 à 90 groupes POE.

17. Composition selon l'une quelconque des revendications précédentes, dans laquelle la phase aqueuse représente de 30 à 60 pour cent en volume de la microémulsion d'eau dans l'huile.

18. Composition selon l'une quelconque des revendications précédentes, dans laquelle la substance active est une protéine ou un peptide.

19. Composition selon l'une quelconque des revendications précédentes, dans laquelle la substance active est un antagoniste du fibrinogène.

20. Composition selon la revendication 19, dans laquelle la substance active est un peptide ayant la séquence cyclo(S,S)-N^{α}-acétyl-Cys-(N^{α}-méthyl)Arg-Gly-Asp-Pen-NH_2.

21. Composition selon l'une quelconque des revendications 1 à 12, dans laquelle la substance active est un peptide de libération d'hormone de croissance.

22. Composition selon la revendication 21, dans laquelle la substance active est un peptide ayant la séquence His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2.

23. Composition selon l'une quelconque des revendications 1 à 11, dans laquelle la substance active est choisie dans le groupe constitué par les calcitonines, les insulines et les hormones de croissance humaines.

24. Composition de microémulsion d'eau dans l'huile biologiquement compatible qui se transforme en une émulsion d'huile dans l'eau par addition d'eau, comprenant:

(a) jusqu'à 60 pour cent en volume d'une phase aqueuse à dispersion interne comprenant une quantité efficace d'une substance soluble dans l'eau, biologiquement active;
(b) de 5 à 99 pour cent en volume d'une phase huileuse continue comprenant au moins une huile acceptable sur le plan pharmaceutique; et
(c) de 1 à 70 pour cent en volume d'un tensioactif ou d'un mélange de tensioactifs comprenant un ou plusieurs acides gras en C_{8-32} ou des sels de ceux-ci, le tensioactif ou le mélange de tensioactifs ayant une BHL d'au moins 7.

25. Composition de microémulsion d'eau dans l'huile selon la revendication 24, dans laquelle la substance biologiquement active est un agent thérapeutique et est une protéine, un peptide, un immunogène, ou une autre substance pharmaceutiquement active et le coefficient de partage eau:huile de la substance active est supérieur à 10:1.

26. Composition de microémulsion d'eau dans l'huile selon la revendication 25, dans laquelle la substance biologiquement active est une protéine ou un peptide.

27. Composition de microémulsion d'eau dans l'huile selon la revendication 24, dans laquelle la phase huileuse comprend un triester de glycérol ayant 20-60 atomes de carbone, un diester en C_{15-40} de propylèneglycol, ou des mélanges de ceux-ci.

28. Composition de microémulsion d'eau dans l'huile selon la revendication 24, comprenant: de 0,1 à 15 pour cent en volume de la phase aqueuse; de 50 à 90% en volume de la phase huileuse continue et de 2 à 50% en volume du tensioactif.

29. La composition selon l'une quelconque des revendications précédentes destinée à être utilisée en thérapie.

30. Utilisation d'une composition de microémulsion d'eau dans l'huile qui se transforme en une émulsion d'huile dans l'eau par addition d'eau, dans la préparation d'un médicament destiné à être utilisé en thérapie, comprenant l'administration d'une quantité efficace du médicament dans l'organisme d'un animal, où l'administration s'effectue par voie parentérale, entérale, ou par l'intermédiaire d'une membrane muqueuse quelconque, dans laquelle la composition de microémulsion comprend:

(a) jusqu'à 60 pour cent en volume, basé sur le volume total de la microémulsion, d'une phase aqueuse à dispersion interne comprenant une quantité efficace d'une substance biologiquement active ayant un coefficient de partage eau:huile supérieur à 10:1;
(b) de 5 à 99 pour cent en volume d'une phase huileuse continue comprenant des diesters de propylèneglycol ayant de 7 à 55 atomes de carbone; et
(c) d'environ 1 à 70 pour cent en volume d'un tensioactif ou d'un mélange de tensioactifs, le tensioactif ou le mélange de tensioactifs ayant une balance hydrophile-lipophile de 7 à 14,

31. Utilisation d'une composition de microémulsion d'eau dans l'huile qui se transforme en une émulsion d'huile dans

l'eau par addition d'eau, dans la préparation d'un médicament destiné à être utilisé en thérapie, comprenant l'administration d'une quantité efficace du médicament dans l'organisme d'un animal, où l'administration s'effectue par voie orale, dans laquelle la composition de microémulsion comprend:

(a) jusqu'à 60 pour cent en volume, basé sur le volume total de la microémulsion, d'une phase aqueuse à dispersion interne comprenant une quantité efficace d'une substance biologiquement active ayant un coefficient de partage eau:huile supérieur à 10:1;
(b) de 5 à 99 pour cent en volume d'une phase huileuse continue comprenant au moins une huile pharmaceutiquement acceptable; et
(c) de 1 à 70 pour cent en volume d'un tensioactif ou d'un mélange de tensioactifs comprenant des monoglycérides en C_{9-13}, des diglycérides en C_{15-23}, et des mélanges de ceux-ci, le tensioactif ou le mélange de tensioactifs ayant une balance hydrophile-lipophile de 7 à 14.

32. Utilisation d'une composition de microémulsion d'eau dans l'huile qui se transforme en une émulsion d'huile dans l'eau par addition d'eau, dans la préparation d'un médicament destiné à être utilisé en thérapie, comprenant l'administration d'une quantité efficace du médicament dans l'organisme d'un animal, où l'administration s'effectue par voie rectale, dans laquelle la composition de microémulsion comprend:

(a) jusqu'à 60 pour cent en volume, basé sur le volume total de la microémulsion, d'une phase aqueuse à dispersion interne comprenant une quantité efficace d'une substance biologiquement active ayant un coefficient de partage eau:huile supérieur à 10:1;
(b) de 5 à 99 pour cent en volume d'une phase huileuse continue; et
(c) de 1 à 70 pour cent en volume d'un tensioactif ou d'un mélange de tensioactifs, le tensioactif ou le mélange de tensioactifs ayant une balance hydrophile-lipophile de 7 à 14.

33. Utilisation d'une composition de microémulsion d'eau dans l'huile dans la préparation d'un médicament destiné à être utilisé en thérapie, comprenant l'administration d'une quantité efficace du médicament dans l'organisme d'un animal, où l'administration s'effectue par voie parentérale, entérale, ou par l'intermédiaire d'une autre membrane muqueuse quelconque, et comprenant en outre la transformation de la microémulsion d'eau dans l'huile en une microémulsion d'huile dans l'eau après l'étape d'administration, par l'addition d'un liquide corporel aqueux, dans laquelle la composition de microémulsion comprend:

(a) jusqu'à 60 pour cent en volume, basé sur le volume total de la microémulsion, d'une phase aqueuse à dispersion interne comprenant une quantité efficace d'une substance biologiquement active ayant un coefficient de partage eau:huile supérieur à 10:1; et un modificateur présent en quantité suffisante pour provoquer la transformation de la microémulsion d'eau dans l'huile en une microémulsion d'huile dans l'eau après addition d'un milieu aqueux;
(b) de 5 à 99 pour cent en volume d'une phase huileuse continue comprenant au moins une huile acceptable sur le plan pharmaceutique; et
(c) de 1 à 70 pour cent en volume d'un tensioactif ou d'un mélange de tensioactifs, le tensioactif ou le mélange de tensioactifs ayant une balance hydrophile-lipophile d'au moins 7.

34. Utilisation selon la revendication 32, dans laquelle la composition est un solide.

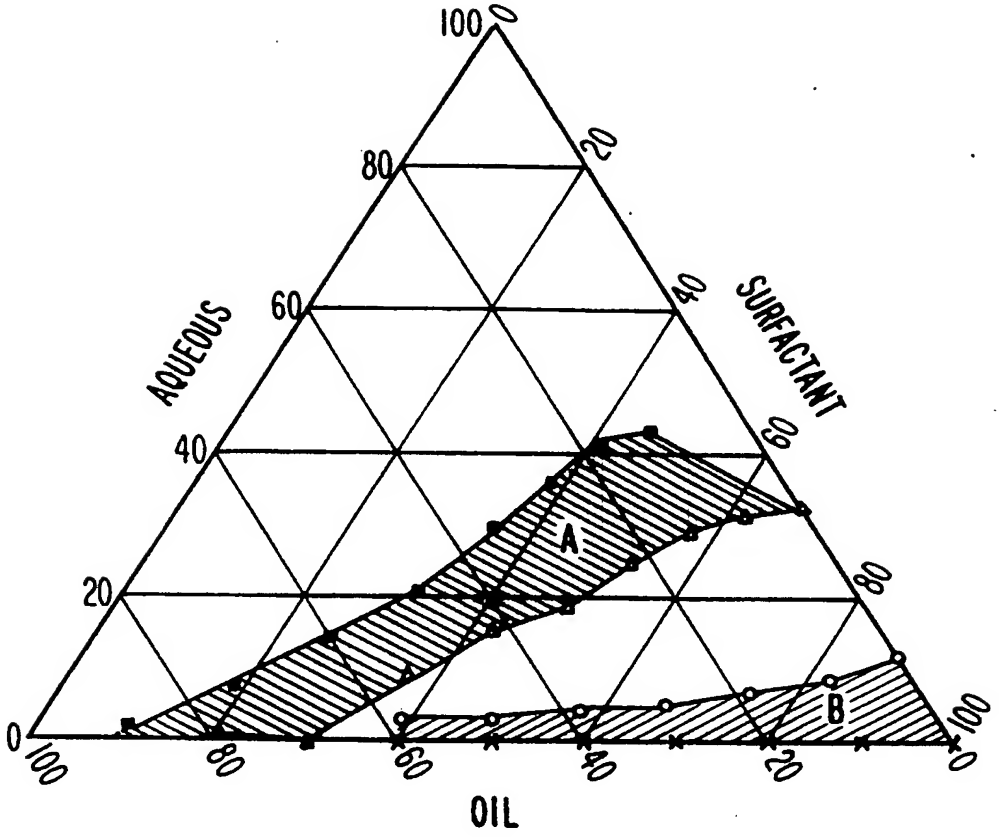
35. Utilisation selon la revendication 30 ou 33, dans laquelle l'administration s'effectue par voie orale.

36. Utilisation selon l'une quelconque des revendications 30, 31, 32 ou 34, comprenant en outre la transformation de la microémulsion d'eau dans l'huile en une microémulsion d'huile dans l'eau.

37. Utilisation selon l'une quelconque des revendications 30, 31, 33, 35 ou 36, dans laquelle la microémulsion d'eau dans l'huile est un liquide.

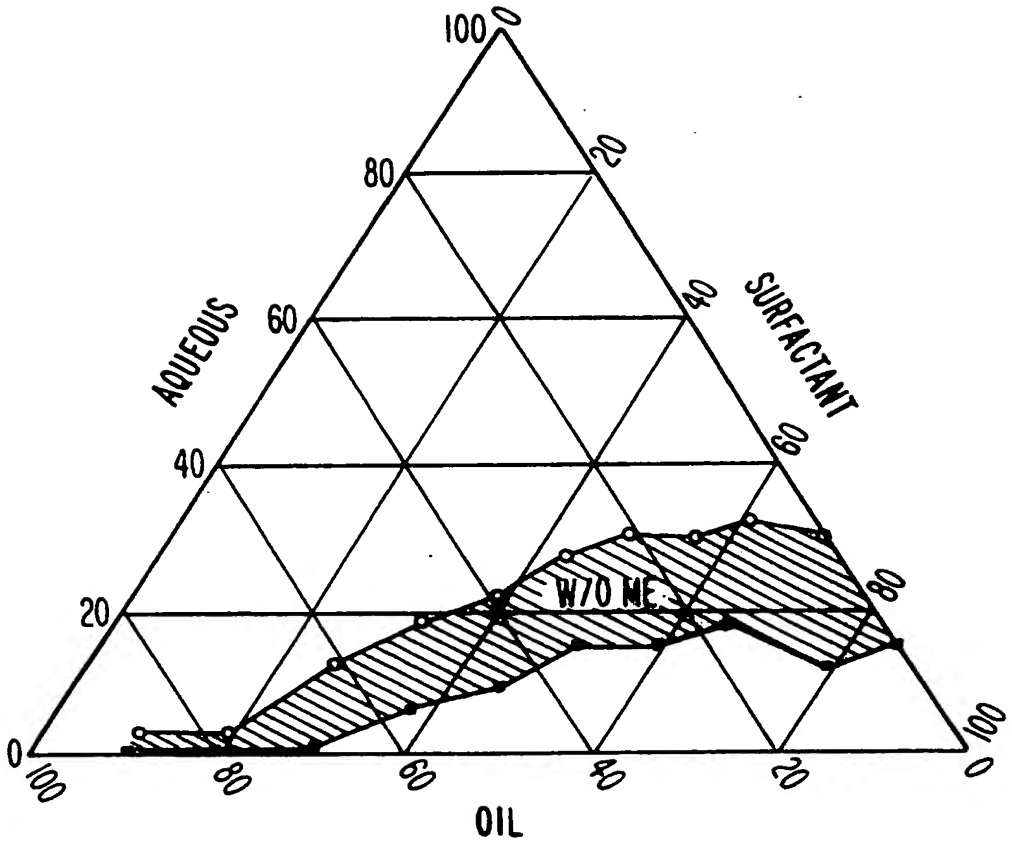
38. Utilisation selon l'une quelconque des revendications 30 à 37, dans laquelle la substance biologiquement active est une protéine thérapeutique ou un peptide.

39. Utilisation selon l'une quelconque des revendications 30 à 38, dans laquelle la substance biologiquement active est un antagoniste du fibrinogène, un peptide de libération d'hormone de croissance, une calcitonine, une insuline ou une hormone de croissance humaine.



■ MAX AQ%(w/w)
○ MAX.MIN AQ%
△ MIN AQ%(w/w)
× MIN.MIN AQ%

Fig. 1



■ MIN Aq%(w/w) ○ MAX AQ%(w/w)

Fig. 2

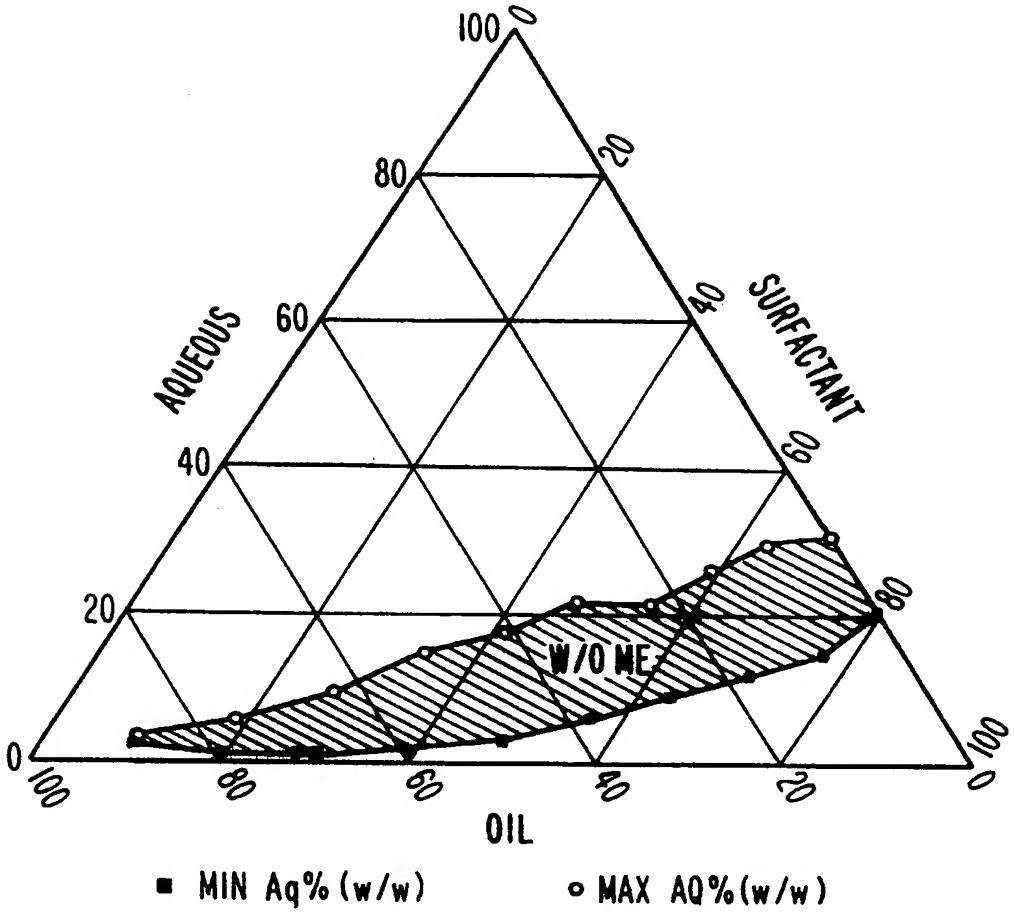
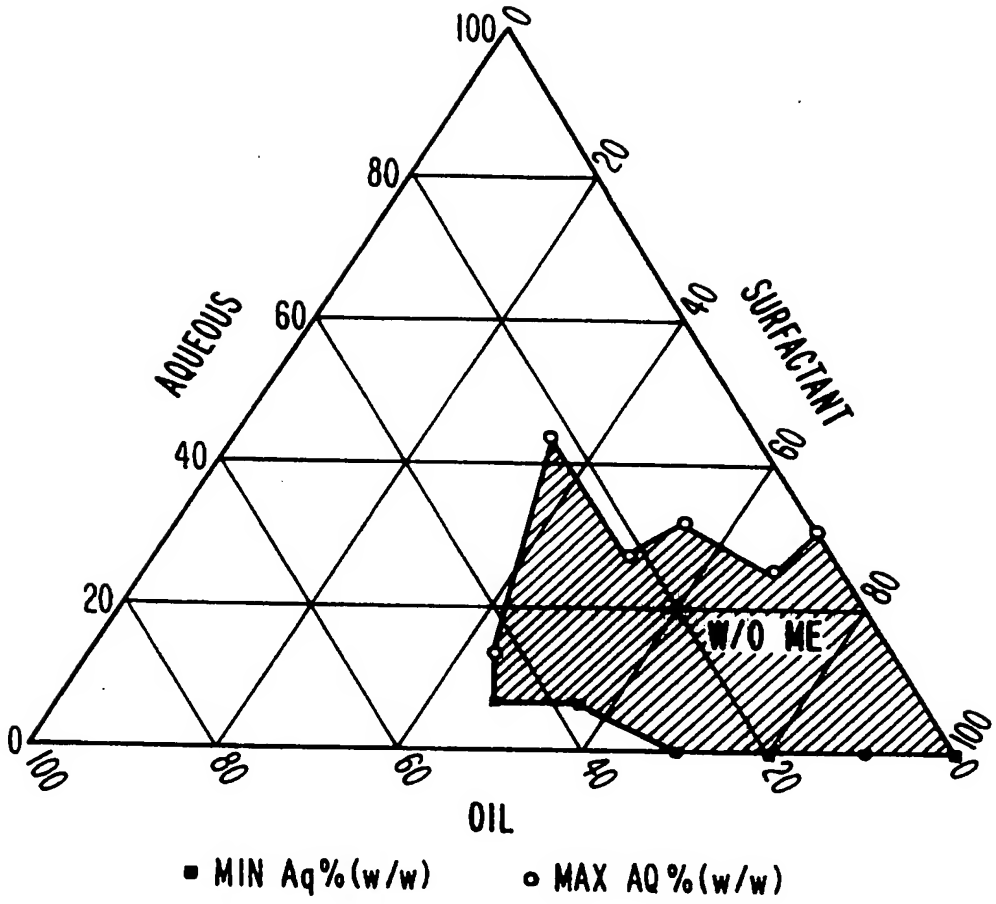

100
0
80
20
60
40
40
60
20
80
0
100
100
80
60
40
20
0
AQUEOUS
SURFACTANT
OIL
W/O ME
■ MIN Aq% (w/w)
○ MAX AQ% (w/w)

Fig. 3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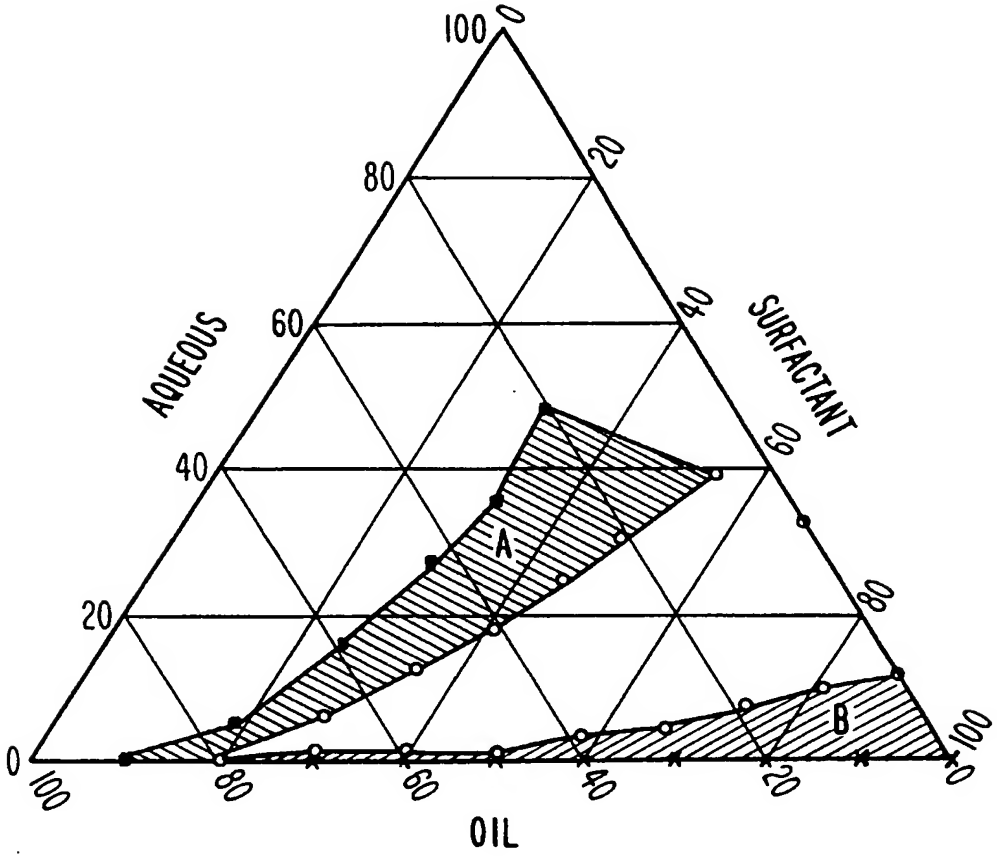
80
0
100
100
80
60
40
20
0
AQUEOUS
SURFACTANT
OIL
W/O ME
■ MIN Aq%(w/w)
o MAX AQ%(w/w)

Fig. 4



■ MAX AQ% (w/w)
○ MIN AQ% (w/w)
○ MAX.MIN AQ%
x MIN.MIN AQ%

Fig. 5